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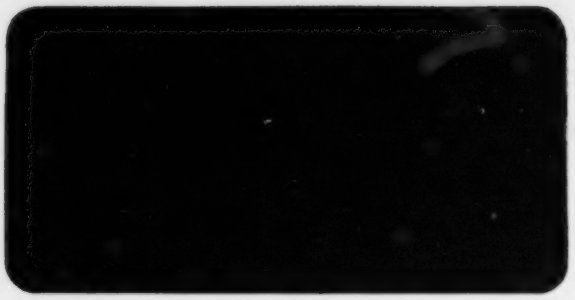

Final Report
by:

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BIOLOGICAL DECARBONIZATION OF SPENT PETROLELUM CATALYSTS UNSOLICITED PROPOSAL UP-C7-009

83 pp

MICROMEDIA

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EXECUTIVE SUMMARY

The objective of this laboratory program was to investigate the use of various microbial cultures to biologically decarbonize spent petroleum catalysts. Despite positive indications in the literature and using different cultures and media formulations, no significant decarbonization was made by the commercially available mixed cultures of microorganisms tested.

RÉSUMÉ DE LA DIRECTION

L'objectif de ce programme de laboratoire était d'étudier l'utilisation de diverses cultures microbiales pour décarburer de facon biologique des catalyseurs de pétrole utilisés. En sus des indications positives affiremées par la litérature et malgré l'utilisation de diverses cultures et diverses compositions de milieu, aucune décarburation importante n'a été obtenue à partir des cultures de miscroorganismes mélangées, qui ont été testées et qui sont disponsibles sur le marché.

1. INTRODUCTION

A contract entitled "Biological Decarbonization of Spent Petroleum Catalysts" (DSS Contract No. 23440-7-9224/01-SQ) was awarded to The Cambrian Engineering Group Limited (TCEGL - an Agra Corporation subsidiary) under the Unsolicited Proposals Program. The scope of work included was originally proposed as a portion of a larger body of work to investigate "The Biological Recovery of Metal Values From Spent Catalysts". The contract awarded called for the subcontracting of work to: Ortech International (OI - formerly The Ontario Research Foundation) of Mississauga, Ontario; Biological Consultants Corp. (BiCo - a former Agra subsidiary), of Trussville, Alabama; and the Saskatchewan Research Council (SRC).

The objective of this laboratory program was to investigate the efficacy of commercially available cultures of microorganisms on the decarbonizing of spent petroleum catalysts. This was with the view to finding an economically viable biological alternative to the current, energy intensive regeneration methods. On successful completion, the next phase of the work would be to leach the residual metals from the decarbonized catalyst using hydrometallurgical or biological means.

The scope of work included the following tasks: 4.1, a Literature Search; 4.2, Catalyst Characterization; 4.3, Feed Pretreatment; 4.4, Comparison of Feed Pretreatments; and 4.5, the Evaluation of the Catalyst.

2. EXPERIMENTAL

The Literature Search, Task 4.1, was designed to find if literature was available to support the hypothesis that mixed cultures of microorganisms are capable of decarbonizing catalysts with the understanding that complex hydrocarbons were likely to be present. Also under investigation was whether indications in the literature were that the metals expected to be present (nickel, cobalt, molybdenum, tungsten, vanadium, chromium, and copper) could be bioassimilated or extracted and were not bioinhibitory. This task was performed by OI and BiCo.

The second task was to characterize the catalyst to be used for the remaining three tasks of this contract. The analysis of the catalyst material included: total Kjeldahl nitrogen, hydrocarbon characterization, total phosphorous, total sulphur, total chloride, total organic carbon, and metal concentrations. OI was responsible for the Catalyst Characterization.

The Feed Pretreatment, Task 4.3, was divided into two parts: the small and large scoping tests. The object of the small scoping tests, performed by BiCo, was to compare the performance of several, commercially available cultures at the decarbonizing of the catalyst. Based on the budget available BiCo chose a gravimetric method for the comparison of the relative performance of the cultures chosen. In addition, BiCo used visual observations and previous experience to decide which culture was most effective at the task at hand.

Once BiCo recommended a particular commercial culture, OI's task, the large scoping tests, was to optimize the growth conditions, nutrients, and other conditions for culture growth. Based on discussions with the scientific authority at that time, OI also investigated the effect of size reduction and the addition of a surfactant on the

microbial growth. Once again, a comparison of the weight of the samples before and after treatment was used to determine the percentage of carbon degraded in each test prepared.

Task 4.4, the Comparison of Feed Pretreatments, also performed by BiCo and OI, ran cocurrently with the analysis for Task 4.3. It was based on the experimental results and observations gathered for that task.

On March 8,1989, TCEGL was advised by the Science Branch Contracting Officer that, based on progress to that date, all work on the project had to be completed by the end of the fiscal year, March 31, 1989. As a result, the large scoping tests were terminated by that date and the Comparison of Feed Pretreatment performed by OI was halted in progress by that date. Also, it was not possible to proceed with the Evaluation of Catalyst (assigned to SRC) since degraded samples of catalyst were not available to them on time for testing to be completed before the end of March.

3. RESULTS

3.1 Task 4.1

The Literature Search performed by OI found that, although difficult, degradation might be possible using a mixed culture of microorganisms due to co-oxidation among other factors. Some of the factors that the literature indicated might be of importance were the presence of N, P, and K nutients as well as emulsifiers and surfactants.

BiCo found that while there was no evidence to suggest that bacteria could not function in the presence of the metals expected, there was also no documentation available on the leaching capabilities of commercially available bacteria formulations. Also, particular bacteria have affinity for certain metals and that they have different "mechanisms with which they can accumulate, agglomerate, adsorb, or physically retain heavy metals in or on their bio mass". BiCo confirmed OI's findings that the culture medium can have a significant effect on the performance of the microorganisms.

A copy of both OI's and BiCo's report on their respective Literature Searches can be found in Appendix A.

3.2 Task 4.2

The Catalyst Characterization found that: approximately 20 per cent of the catalyst sample was carbon; of the hydrocarbons present 45.6 per cent were aliphatic, 36.0 per cent were polyaromatic hydrocarbons, 6.3 per cent were sulphur-containing polyaromatic hydrocarbons, and 12.1 per cent were unresolved hydrocarbons; that

the metals present in significant amounts were vanadium, nickel, molybdenum, and cobalt; and that the nitrogen, phosphorous and chloride concentrations were very low.

The characterization performed at that stage did not distinguish between graphitic carbon and carbon which is in a biologically useful form. A copy of the report for this task is included in Appendix B.

3.3 Task 4.3

BiCo found that the commercially available formulations which they tested were able to accomplish little or no degradation of the hydrocarbons present. However, the results of their experimentation suggested that much of the carbon present was in the graphitic form or that the graphitic carbon was "masking" the organic present making it difficult for the bacteria to act upon it. Based on visual observations and their previous experience with the cultures used, BiCo recommended the use of an Advanced Bio Cultures formulation, L-104, for the large scoping tests to be carried out by OI. A copy of BiCo' report is included in Appendix C.

Due to the concerns raised by BiCo about the percentage of graphitic carbon present in the catalyst sample, OI performed a soxhlet extraction on catalyst sample. It was found that only approximately 20 per cent of the total carbon is not graphitic in form.

The variable which OI used in order to attempt to optimize the culture growth were: attrition of the sample before culturing; variation in the mineral salts used to aid microbial growth; and addition of a surfactant. The results from the biodegradation were compared to the weight loss recorded for samples which were subjected to

The variable which OI used in order to attempt to optimize the culture growth were: attrition of the sample before culturing; variation in the mineral salts used to aid microbial growth; and addition of a surfactant. The results from the biodegradation were compared to the weight loss recorded for samples which were subjected to high temperatures as in the conventional regeneration process. There was no apparent pattern to the weight losses which were recorded for the various treatment methods. A copy of OI's report is included in Appendix C.

3.4 Task 4.4

BiCo found, in comparing feed pretreatments, that formulation L-104 was the most effective at degrading the carbonization poisoning the catalyst sample. OI found that the different treatments tested did not produce results which had any significant variance.

3.5 Task 4.5

This task was not performed.

4. DISCUSSION

There was considerable difficulty in obtaining a suitable sample for this program of experimentation. Firstly, it was desired to find a sample which could be used for the metals recovery phase should Phase I be successful. However, these metals normally are found in the highest boiling point fractions of crude oil and, at the time when this project was started, world prices for oil were such that it was uneconomical for refineries to treat these fractions. As such there was concern that any catalyst samples from an on-stream process would not have the required contamination.

It was arranged that a sample of catalyst could be provided by a local refinery. When the sample was received, it turned out to be of an FCC catalyst which had already passed through the regenerator and was therefor unsuitable for the decarbonization experiments. It was retained, however, in anticipation of work on the metals recovery phase. In the interests of expediency, it was decided that the small and large scoping tests be performed on a hydrotreating catalyst sample which OI had on hand. This sample was only approximately 5 kilograms in weight and placed some restrictions on the number of different tests which could be run.

Originally, BiCo had estimated that their portion of Task 4.3 (the small scoping tests) would take 30 to 60 days. The sample of catalyst was couriered to BiCo on August 12, 1988 and a report on the small scoping tests was not received by TCEGL until January, 1989. Due to the delays which had been experienced, the small scoping tests of Task 4.3 had to be started before the Catalyst Characterization (Task 4.2) was completed.

The results of Task 4.2 were not reported by OI until the beginning of November, 1988 and in any case those results did not differentiate between organic and graphitic carbon. These factors, no doubt contributed to the time taken by BiCo to

complete the small scoping tests. However, once the laboratory tests were completed, it was another month and a half before the report was submitted by BiCo to TCEGL.

In the mean time, OI had some difficulty with equipment scheduling due to the delays in the start of the large scoping tests. As a result, they were not able to make much progress on the large scoping tests until March, 1989.

5. CONCLUSIONS

From the tests performed, it is concluded that the microbial formulations tested were not able to effect a significant decarbonization of the spent petroleum catalyst sample tested. Although it is possible that there are, as suggested by BiCo, OI, and the Literature Search, non-commercial microorganisms such as those found in refinery sludge ponds which can effect the decarbonization required, our scope of work was limited to commercially available cultures.

APPENDIX A

Biological Decarbonization of Spent Petroleum CATALYSTS
(DSS File No. 23440-7-9224/01-SQ)

Phase 1 - Task 4.1
Literature Search; Report

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Biological Decarbonization of Spent Petroleum CATALYSTS
For Cambrian Engineering Group Ltd.

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Biological Decarbonization of Spent Petroleum CATALYSTS
For Cambrian Engineering Group Ltd.

Microorganisms are capable of changing innocuous or hazardous compounds into ones with less harmful potential. Mechanisms of change include mineralization or the complete conversion of an organic molecule into CO_2; co-oxidation or the oxidation of a particular compound (without the usual harvest of metabolic energy) when grown on a substrate compound; and conversion of a substrate molecule into a more oxidized form (e.g. fatty acids). Compounds that resist biotransformation are referred to as recalcitrant or refractory while other compounds may be toxic or inhibitory. It is these compounds which may require special treatment to facilitate biotransformation.

FCC CATALYST

To obtain highly volatile petroleum fractions from crude oil, the oil is processed thermally using a fluid catalytic cracking (FCC) catalyst. A disadvantage of these is that the catalyst becomes hydrocarbon-contaminated and must be regenerated. It is therefore in our interest to develop a process to biologically aid in the degradation of the coke-like residue. Not only are there coke deposits but a wide range of metals including Ca, Na, Cu, V, Fe, Cr, Zn, Ni may also be present (Ryland et al 1960) depending on the nature of the crude oil being processed. The nature of the coke deposits is unknown at present, but once chemically characterized will provide us with an idea of the potential for biodegradation.

In order to assess the potential for carbon biodegradation the project must be researched from a wide source of references. Crude oil is composed of many different fractions with varying degrees of biodegradability. The heavier fractions of oil have generally been more difficult to biodegrade with the tar and asphaltene fractions exhibiting considerable recalcitrance (Jack 1985), while the lighter fractions consisting of the saturated hydrocarbons (n-alkanes) are quite amenable to biodegradation.

ENVIRONMENTAL STUDIES

Many studies have been performed in the environment, ranging from marine to rivers and even in crude oil reservoirs. A review by Connan (1986) described the biodegradation of crude oil in a reservoir as being an oxidative process resulting in α- and β-oxidation, ring cleavage etc. to produce either CO_2 or fatty acid derivitives as metabolic end products. A sequence of degradation increasingly complex structures (i.e. n-alkanes > iso- and anteiso alkanes > alkyl benzenes) had all saturated and aromatic long alkyl chains disappearing first, then branched structures, then polyaromatics, with substituted polyaromatic hydrocarbons (PAH's) being the most resistant. Essential to reservoir crude biodegradation is the presence of flowing water, oil-water contact, oxygen, nutrient supply, and temperature suited to microorganisms. Analysis of hydrocarbons (HC's) persisting in a tundra environment was monitored over 7 years (Atlas et al, 1980). Although microbial numbers increased, HC's as simple as n-paraffins persisted to some degree, with the conclusion that low temperature, O_2 exchange, and nitrogen source were the limiting factors. A general methodology was described for biodegradation of crude in a marine environment by Ballerini and Vandecasteele (1982). They found that asphaltenes and resins were very recalcitrant while the saturated fraction disappeared between 0-20 hours and the aromatics began to disappear between 20-46 hours, but only partially oxidized. An alteration of the marine-like environment in the lab required enrichment with nitrogen (N), phosphorous (P) and reduced iron (Fe^{2+}) using microbes that were isolated from natural polluted waters. Bacteria identified belonged to the Pseudomonas, Moraxella, Acinetobacter and Flavobacterium genus, with Pseudomonas predominating over time.

LABORATORY STUDIES

Most studies have been performed in the laboratory with the intent of transferring the technology to the polluted environment. Walker et al (1975) subjected Louisiana crude oil to a mixture of polluted estuarial bacteria to follow the progress of biodegradation using computerized low-resolution mass spectrometry. Total normal and branched alkanes were 96% degraded (saturates 83%, aromatics 74%) while asphaltenes and resins increased by 28% after degradation, thought to be due to microbial production of extracellular compounds. Susceptibility of aromatics decreased with increasing ring number, and sulfur containing aromatics were extremely resistant. In a related study Walker and Colwell (1976) observed wax formation during microbial degradation of a crude oil. It was noted that weathering could remove the C9-C11 fraction and biodegradation the <C30 fraction, while many long chain n-alkanes >C30 remained as tar balls. Walker et al (1976) also monitored rates of individual components of crude oil using marine sediment organisms. Of the inoculated 80 mg (0.1% v/v) crude oil, a biodegradation rate of 0.18 mg per day was observed with the most consistent decreases in the saturate fraction (86%, 58%, 17% over 0-7, 8-21, 22-49 days respectively). Aromatics resins and asphaltenes decreased during the first two weeks then steadily increased over the next 5 weeks.

The composition of crude oil was determined as important in biodegradability. (Westlake et al 1974). The presence of a normal n-saturate fraction (38%) as well as other unidentified compounds (perhaps the N-S-O containing fraction) in crude, allowed for maximum growth and biodegradability by soil bacteria as compared to an abnormal (30%) n-alkane composition. Phytane and pristane, 2 relatively recalcitrant compounds, were not used by organisms growing in the abnormal n-alkane fraction crude or at 4°C on any of the oils used, but were used at 30°C growing in the normal n-alkane crude.

NUTRIENT SUPPLEMENTS

Nutrient limitations in crude oil or polluted environments, especially N, P and K, generally are rate limiting factors during HC biodegradation. Atlas and Bartha (1972) found that crude oil in unamended sea water (1% v/v) resulted in only 4% (3% biodegraded; 1% mineralization) total biodegradation over 18 days. By adding nitrate [10^{-2}M] and phosphate [3.5×10^{-4}M] together, they achieved rates of 70% biodegradation and 40% mineralization although added separately produced rates comparable to the unamended sea water. Because inorganic N and P are insoluble in the HC phase, Atlas and Bartha (1973) investigated oleophilic N and P fertilizers on an indigenous sea water population of microorganisms. Compared to 5% mineralization in unamended Swedish crude oil media, oleophilic and water soluble N and P provided for observed increases. In the laboratory, Sun Oil CRNF (slow release paraffinized urea) was as good or better than water soluble KNO_3 (70% oil biodegraded) which was twice as good as dodecylurea (35%) or trimethylenetetraurea (15%) in the presence of a suitable P-source. Organic phosphate sources octylphosphate or isooctylphosphate in the presence of inorganic N supported significantly more rapid oil mineralization than inorganic P. A combined oleophilic N and P source hexamethylphosphoramide performed poorly. Using a unique flow through design for use in open seawater, the CRNF and octylphosphate provided the greatest oil biodegradability, although KNO_3 and Na_2HPO_4 provided an initial burst of mineralization due to the washout probably experienced.

Fedorak and Westlake (1981, 1984) reported on the effects of supplementation of N and P on biodegration of aromatics, saturates and sulfur-heterocycles by greenhouse soil microorganisms. The N and P additions resulted in degradation of the n-alkanes (C_{10}-C27 except the isoprenoids) in less than 4 days and aromatics with increasing complexity of structure in 11 days. N-alkanes were degraded without N and P additions, but a 27 day incubation period was required for initiation of degradation of the aromatics, and was proportional to the complexity of the aromatic structure (i.e. napthalene > dimethylnapthalene > phenanthrene > methylphenanthrene). Without N and P additions S-containing heterocycles are degraded after 27 days, however, not to the extent of supplemented media. With N and P additions more extensive degradation of C_2- dibenzothiophenes and some C_3-dibenzothiophenes degradation occurred (no C_3- in absence of supplements).

Carbon to nitrogen ratios (C:N) were analyzed in the degradation of 2 oily sludges in soil (Brown et al, 1983). When the C:N was decreased from 78:1 to 9:1 in the refinery sludge there was 30% greater CO_2 evolution, while similar results were obtained with a petrochemical sludge by decreasing the C:N from 380:1 to 124:1. Excessive amounts of mineral nutrients provided no further benefits, nor did P or K added separately.

Stimulation of indigenous microbes using slow release fertilizer to biodegrade oil on railway track ballast was the subject of a paper by Smith et al (1983). Sulfur coated urea applications resulted in dramatic increases in microbial populations (10^5 mL^{-1} to 4 x 10^7 mL^{-1}) and decreases in extractable oil (saturates reduced considerably with and without fertilizer, aromatics remained stable and polar compounds increased). Oil loadings in the ballast were down 65% in fertilized models compared to controls without fertilizer. Boyles (1984) attempted to create optimal and reproducible conditions necessary for oil biodegradation. A commercial bacterial formulation "Petrobac" (Worne Biochemicals), as well as a defined nutrient solution were used to degrade 50 g oil per litre medium. Results were similar to others presented with rapid n-alkane degradation (C12-C36) occurring <20 hours, napthene and 1 and 2 ring aromatics undergoing rapid degradation initially then much slower after 20 hours and the other classes degraded very slowly in the order isoalkanes > S-aromatics > 3-4 ring aromatics. Polar residues accumulated at the expense of decreasing HC content with final (>2 weeks) degradation products appearing as 4% (of the original weight oil) CO_2, an unquantified cell biomass and the polar residues.

Berwick (1984) investigated the physical and chemical conditions necessary for microbial oil degradation. Bacterial attachment to oil droplets was considered to be crucial to success. Oil dispersion increased as biomass increased, and, that after 5 days while 37% of the oil was attached to bacteria, 46% of the total oil in culture had been degraded. Without nutrient additions, a pure culture Bacillus sp. was shown to degrade higher percentages of individual fractions of crude than a mixed culture of Pseudomonads, although the opposite was shown for oil degradation with nutrient additions. Using 3 g/L diammonium hydrogen phosphate, $(NH4)_2HPO_4$, at 20°C, 56% oil had been degraded by the mixed culture in batch. A rate of 120 mg oil per litre per hour was obtained in a fed-batch system (21.4 gram batches) HC profiles after degradation revealed that the combined wax and saturate fractions were more biodegradable than the aromatics which were more so than the asphalts.

Biological Decarbonization of Spent Petroleum CATALYSTS
For Cambrian Engineering Group Ltd.

MODEL HYDROCARBON DEGRADATION

Using specific hydrocarbons for degradation studies has usually been done with a specific purpose. For example, using recalcitrant HC's to find out exactly why they do or don't get metabolized, or, alternatively, what the metabolic products are of a specific HC fermentation.

Bacteria isolated from a creosote waste pond (Roberts and Hedrick 1979), tentatively identified as Pseudomonas sp. and Alcaligenes sp. were capable of maximum 40% reduction of the phenolic compounds present in creosote. While creosote is commonly used to preserve wood against decay by microorganisms, it is noteworthy that there are some bacteria capable of degradation of this toxic substance. Model recalcitrant HC's were subjected to pure cultures and mixed cultures to determine the amount of biodegradation due to the phenomenon of co-oxidation. Griffin and Cooney (1979) investigated the fate of 4 recalcitrant compounds: pristane (C17), tetradecadiene (TDD), cyclohexane and benzene. Six percent of the pure cultures and 40% of the mixed were able to degrade TDD, 46% pure and 63% mixed culture were able to degrade pristane , indicating the contribution mixed cultures have in the degradation of these 2 compounds. Only 1 of 35 isolates could grow on benzene while no cultures grew on cyclohexane. Co-oxidation, however, was present in 4 of the bacteria and 2 of the fungi when benzene and cyclohexane were analyzed by thin-layer chromotography. These results are significant expeciallly when considering mixed populations, where the co-oxidation of one particularly recalcitrant compound by one organism may result in a compound suited to another organism's metabolism.

The susceptibility to microbial degradation of polycyclic aromatic hydrocarbons (PAH's) containing > 4 rings was shown to occur using a sediment isolated bacterium. (Heitkamp and Cerniglia, 1988). The gram positive rod was capable of mineralizing fluoranthene (90%) and pyrene (63%), both 4 ringed PAH's, as well as small amounts of 1-nitropyrene (12%), 3-methylcholanthene (1.6%) and 6-nitrochrysene (2%) after 2 weeks, while mineralization of benzo-a-pyrene was undetectable. Growth, however, did not occur without the supplementation of the media with common organic carbon sources such as peptone, yeast extract and starch. An interesting study by Vazquez-Duhalt and Greppin (1986) examines the toxicity and fate of heavy metals during bacterial degradation of used motor oil. Used motor oil contains an unusually high concentration of PAH's due to their high temperature of combustion, as well as an increased level of metals (Pb, Zn, Cu, Ni, Cr, Cd). The bacterium Pseudomonas fluorescens was not adversely affected by the metal concentration and was able to metabolize the oil mostly to long and medium chain fatty acid residues. The metals in the oil phase ended up either solublized in the aqueous phase or adsorbed and assimilated by the bacterium.

COAL BIODEGRADATION

Coal biodegradation has ultimately become a study in methods of liquification to produce cleaner fuel products. A complex HC chemistry includes dibenzothiophenes, biphenyls, fused aromatics with reactive side groups of amines and carboxyl groups. Lignin biodegradation is often used as a model due to the many lignin-like structures (polyphenyl-propanoid units) in coal (Olson et al, 1986).

Biological Decarbonization of Spent Petroleum CATALYSTS
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Liquification of coal has been shown to be possible with a variety of organisms, chiefly fungi, although bacteria may aid in the removel of carboxyl groups and other reactives. Polyporous versicolor was demonstrated by Cohen and Gabrielle (1982) to liquify coal but Olson et al (1986) were unable to obtain similar reproducible results. The microbial solublization of coal was followed by Scott et al (1986) using a variety of fungi. The screening tests used surface cultures of mycelium in which coal particles were placed and observed for liquification. Following a 2-3 day lag, small liquid droplets began to appear with the completion of liquification occurring in 7-14 days. Six fungal isolates of various sources were described as at least solublizing 1 type of coal (of 6 different types) with the Candida sp., Penicillium waksmanii, and Sporothrix sp. being the most versatile. Coal samples which had undergone a chemical oxidation (e.g. HNO_3, ozone) were much more susceptible to liquification. In trying to determine the best selection procedure for bacterial isolates capable of degrading bituminous coal Munoz Cebrian (1983) inoculated soils with various HC substrates. The highest degree of selectivity resulted from lubricating oil, asphalt cement and crude oil inoculated soils. Also, those bacteria, for example Mycobacterium and Nocardia, which were selected for and isolated with asphalt cements produced the greatest effect on the bituminous materials, which by nature were similar to the treatment products.

MICROORGANISMS

The identification of specific microorganisms able to degrade hydrocarbons has occupied many workers both for practicality and scientific interest. A complete listing of all the microorganisms capable of HC degradation would be too extensive but some generalizations may be made. Einsele and Fiechter (1971) did a survey of popular microbes to find that of the yeast, Candida sp. probably has the most economic importance, with C maltosa and C. lipolytica able to grow on aromatic HC's (Cnow and Bell 1981). Bacterial families include Pseudomonaceae, Enterobacteriaceae, Micrococcaceae, and Bacillaceae, the first group of which comprises the most cited examples. Fungi are numerous but some examples are Aspergillis, Penicillium, Fusarium and Cladosporium.

Three bacteria from sea water (Soli and Bens, 1972) and identified as Corynebacterium sp. Arthrobacter sp. and Achromobacter sp. were able to utilize HC's as well as destroy 50% of a crude oil in 48 hours in a mixed culture. Atlas and Bartha (1972) used a Flavobacterium sp. and a Brevibacterium sp. to degrade both paraffinic crude oil and a defined HC mixture but found that mixed cultures provided greater biodegradation. Other microorganisms are described throughout this review.

EMULSIFIERS AND SURFACTANTS

Emulfifiers and surface active agents have the distinct property of increasing the interface between the HC and aqueous phase allowing greater access of the HC substrate to the microbes. Often the emulsifier is produced by an organism growing on the HC substrate with a biodegradation rate limiting factor being the stability and average droplet size of the emulsion. An emulsifying agent was described by Holdom and Turner (1969) as isolated from Mycobacterium rhodochrous grown on n-decane which, as a non-ionic detergent class of surfactant did not compare as well to some commercial detergents with surfactant properties (Na-lauryl sulfonate, Lubrol W, Teepol 10). Zajic and Supplison (1972) working with mixed culture isolated from sewage (bacteria and fungi) and growing on #6 fuel oil noted the emulsification of mineral salts media within 24-48 hours. Particularly interesting was the fact that freeze-dried biomass (in quantities 0.003% - 0-1%(W/W) with 1% w/v fuel oil as substrate) was able to emulsify fuel oil in mineral salts media very effectively. Further investigation (Zajic et al, 1974) led to a characterization of the emulsifier as being synthesized from the paraffinic component of oil, chemically a high-molecular weight polysaccharide and able to produce oil globules 3-16 uM in size. Panchal and Zajic (1977) have also isolated emulsifying factor from Corynebacterium sp. with maximum production when grown on kerosene or n-hexadecane. Defined media was required to obtain maximum surfactant yield from 2 bacteria isolated from natural asphalt deposits, both of which had a specific period in the growth cycle that maximum production occurred (Gerson and Zajic 1977). Other important factors concerning emulsifiers are the:

1) pH Maxima-minima; the absence of divalent cations at pH>7 or the protonation of carboxyl groups at pH<4 reduced or inhibited emulsification (Rosenberg et al 1977); pH optimum 2-5 for Candida lipolytica (Cirigliano and Carman, 1984)

2) presence of cyclic (aromatics and non-aromatics) and aliphatic (straight or branched) hydrocarbons for effective emulsification (Rosenberg et al 1977)

3) optimal biosurfactant concentration; 1.6 g biosurfactant/Kg petroleum (Mattei and Bertrand, 1985), 0.3% w/v (Panchal and Zajic, 1977), also related to the critical micelle concentration (CMC) the point at which the biosurfactant begin to aggregate and form micelles.

BIOREACTORS

The design of the bioreactor and subsequent process will certainly have an effect on the economics and success of the project. The use of venturi aeration in a HC-degrading bioreactor was implemented in pilot trials by Berwick (1985). In a constant volume of 1.18 m^3, a mixed population from oil contaminated marine mud in Bushell-Haas mineral salts media (Difco) biodegraded crude oil at a rate of 0.168 Kg oil/m^3/day with >95% degradation of all fractions. Heat transfer was deemed to be a major obstacle, while foaming (10 g oil/L) and expanded oil (100 g oil/L) were problems deemed to be suitable to emulsification to aid in dispersal.

A cyclone fermentor as designed by Liu et al (1981) was made so as to accomodate aerobic or anaerobic processes. In this enclosure, the gas flow in and out is monitored with high accuracy to obtain mass balance calculations. The advantages seen with this system are the negligable loss of compounds or biomass due to "peripheral rings" produced by stagnant solutions, and the relative inexpense of the design (<$500.00).

Biological Decarbonization of Spent Petroleum CATALYSTS
For Cambrian Engineering Group Ltd.

An extended activated sludge process was designed to degrade waste oil in coolant fluids (Davis, 1978). Control of pH, temperature, dissolved oxygen concentration (DOC), and nutrient concentration was critical in preventing bulking (bacterial mass "floc" floats to surface) but by monitoring the chemical oxygen demand, the reactor had an efficiency of >92%.

Bertrand et al (1983) developed a continuous culture technique for the biodegradation of crude oil in sea water. Using non-aseptic methods and a mixed bacterial population, a 20 litre fermentor was implemented, stirring at 750 rpm. It was found that crude oil biodegradation was absent without previous emulsification. Optimal flow rates (D^{-1}) and substrate concentrations [S] were deemed to be 0.1 hr^{-1} and 6 g/L which gave maximum 60% degradation. If the D^{-1} or [S] was increased, less degradation would occur but the rate increased.

Two concepts of bioreactors have been envisioned for coal solublization (Scott et al, 1986). A gas-phase-continuous fermentation in a fixed bed reactor is based on surface myceluim culture experiments. Here a constant flow of nutrients is allowed to trickle down fixed coal particles with the fungi adhered to the particles. Fresh coal may be added as necessary. A fluidized bed reactor is based on suspension culture experiments and involves the upflow of nutrients and microorganisms.

Witte, Hoppe, McCready (1986) have had success in degrading carbonnaceous material present in a Cu-Mo concentrate using biosurfactant production as the main method of removal. In an 1100 L leach tank with 8% slurry of solids, all but 15% of the carbonnaceous material was removed. Very little mineralization, however, occurred. Regeneration of the bacterial population was performed 10 days after initiation of the process by decantation and thereafter every 48 hours.

ANALYSIS OF BIODEGRADATION

To follow the progression and initiation of biodegradation of the FCC catalyst, many methods are available including gas chromatography, HPLC and mass spectrometry but expense dictates more moderate methods be considered.

The AFNOR biodegradability screening test is based on production of CO_2 (or related metabolic process) by the organisms in culture. In a chemically defined medium, the flasks of varying composition (test chemical and/or glucose and/or inoculum) are allowed to grow for a specified time period. Using proper controls, the toxicity and or biodegradability of a compound is determined (Liu 1986).

Following the kinetics of the biodegradability has potential in realizing the maximum growth rates at varying substrate concentrations. With physical and chemical variables constant (pH, temperature, DOC, etc), the bacterial growth rate may be monitored by relative plate counts. With increasing substrate, the growth rate will become the limiting factor where the relationship $u = um [S]/Ks + [S]$, (where u = the growth rate, um = maximum growth rate, [S] = substrate concentration and Ks = [S] at 1/2 um), if put into the reciprocal form represents the straight line $y = ax + b$. By following simple calculations and analytical procedures, progress of the system may be realized (Liu 1986).

CONCLUSION

The presumed harsh nature of the HC contaminated FCC catalyst will probably require considerable biodegradation. Although anaerobic processes cannot be ruled out, the aerobic process contributes so much more to degradation due to the nature of the oxidative requirements. Most reports have stated the particular recalcitrance of aromatics, resins and asphaltenes while the presence of n-alkanes in the crude oil allows for a more complete biodegradation. The presence of the N, P, and K nutrients contribute to a controlled complete process. Depending on the nature of the process these nutrients may be the difference in recalcitrant compound breakdown.

Mixed populations of microorganisms usually have an advantage over pure cultures, especially when dealing with recalcitrants. The co-oxidation of one compound, altering it slightly, may provide another organism with substrate and further down the line to CO_2. Coal biodegradation may be the most parallel example of what we are dealing with. Liquification has been accomplished by a variety of fungi, a process which could prove to our advantage. To isolate organisms capable of HC biodegradation, a suitable starting point will be chronically contaminated soil, as the population there will be acclimatized to various HC's. Alternatively existing proven HC degraders may be acquired. Important in the selection of microorganisms will be the capability to produce emulsifiers that increase the interface between the aqueous-hydrocarbon-microorganism. If not this, then commercial surfactants may prove necessary.

Various bioreactors have been successfully implemented with most problems arising from heat emulfification and substrate form. Analysis of project progression will depend on the nature of the initial successes. Optimization of the process may be made easier using the AFNOR system or kinetic modelling.

REFERENCES

Atlas R.M., Bartha R. (1972) "Degradation and Mineralization of Petroleum by Two Bacteria Isolated from Coastal Waters". Biotech Bioeng 14, pp 297.

Atlas R.M., Bartha R. (1972a) "Degradation and Mineralization in Sea Water: Limitation by Nitrogen and Phosphorous". Biotech Bioeng Vol. 14 pp 309-318.

Atlas R.M., Bartha R. (1973) "Stimulated Biodegradation of Oil Slicks_ Using Oleophilic Fertilizers". Env.Sci.Tech 7 (6) pp 538-541.

Atlas R.M., Sexstone A. Gustin P. (1980) "Biodegradation of Crude Oil by Tundra Soil Microorganisms" in Biodeterioration, Oxley, Allsop, BEcker (eds) Pittman, London (1980) p 21-28.

Ballerini D. Vandecasteele J. P. (1982) "Biodegradation of Crude Oil in a Marine Environment - General Methodology" in Energy and Environmental Chemistry, ed L.H. Keith, Ann Arbor Science, pp 185-197.

Bertrand J.C., Rambeloarisoa E., Rontani J.F., Guisti G., Matteri G. (1983) "Microbiol Degradation of Crude Oil in Sea Water in Continuous Culture". Biotech Letters 5 (8), 567-572.

Berwick P.G. (1984) "Physical and Chemical Conditions for Microbial Oil Degradation" Biotech. Bioeng 25 pp 294-1305.

Berwick P.G. (1985) "Pilot Trials of the Microbiol Degradation of Christos - Bitas Water in Oil Emulsion (Chocolate Mousse) and BP Llandarcy Gas Oil using Venturi Aeration", Biotech Bioeng 27, pp 94-108

Boyles D.T.A., (1984) "Biodegradation of Topped Kuwait Crude" Biotech Letters 6. 31-36.

Brown K.W., Donnelly K.C., Devel Jr. L.E. (1983) "Effects of Mineral Nutrients Sludge Application Rate, and Application Frequency on Biodeterioration of Two Oily Sludges". Microb Ecol 9, 363-373.

Cohen M.S., Gabrielle P.D. (1982) "Degradation of coal by the fungi Polyporus versicolor and Poria monticola" Appl. Environ Microbiol. 43, 23-27.

Connan J. (1986) "Biodegradation of Crude Oils in Reservoirs" in Microbiol Problems in the Offshore Oil Industry. Proceedings of an International Conference, pp 49-55 Aberdeen, April 1986 Ed. Hill, Shennan, Watkins.

Crow S.A., Bell S.L. (1981), "Effects of Aromatic Hydrocarbons on Growth of Candida maltosa and Candida lipolytica". DevInd Microbiol 22, , 437-442.

Davis R.M. (1978) "Biodegradation of High Level Oil in Water Emulsion" Biotech Bioeng Symp No. 8, pp 415-422.

Einsele A., Fiechter A. (1971) "Liquid and Solid Hydrocarbons" Adv. Biochem. Engineering 1.

Fedorak P.M., Westlake D.W.S. (1981) "Degradation of Aromatics and Saturates in Crude Oil by Soil Enrichments" Water, Air, and Soil Pollution 16, 367-375.

Fedorak P.M., Westlake DWS (1984) "Degradation of Sulfur Heterocycles in Prudhoe Bay Crude Oil by Soil Enrichments". Water, Air, and Soil Pollution 21, 225-230.

Gerson D.F., Zajic J.E. (1977) "Surfactant Production from Hydrocarbons by Corynebacterium lepus sp. nov. and Pseudomonas asphaltenicus sp. nov." Dev. Ind Microbiol. 19, 577-599.

Griffin W.M., Cooney J.J. (1978) "Degradation of Model Recalcitrant Hydrocarbons by Microorganisms from Freshwater Ecosystems". Dev. Ind. Microbiol. 20, 479-488.

Heitkamp M.A., Cerniglia C.E. (1988) "Mineralization of Polycyclic Aromatic Hydrocarbons by a Bacterium Isolated from Sediment below an Oil Field" Appl. Env. Microbiol. 54 (6) pp 1612-1614.

Holdom R.S., Turner A.G. (1969) "Growth of Mycobacterium rhodochrous on n-decane: a new growth factor and Emulsifying agent". J. Appl. Bact. 34 pp 448-456.

Jack T.R. (1985) "Application of Bacteria to insita recovery of heavy oil". 3rd International Conference on Heavy Crude and Tar Sands, Long Beach, California, Volume 2, 1985. UNITAR/UNDP Information Centre for Heavy Crude and Tar Sands.

Liu D., Strachan W.M.J., Thomson K., Kwasniewska K. (1981) "Determination of the Biodegradability of Organic Compounds, "Env. Sci Tech. 15, pp 788.

Liu D. (1986) "Biodegradation Tests" in Toxicity Testing using Microorganisms Vol 2 ed B.J. Dutka, G. Bitton, CRC.

Masters M.J., Zajic J.E. (1970) "Myxotrophic Growth of Algae on Hydrocarbon Substrates" Dev Ind Microbiol. 12 77-86.

Mattei G., Bertrand J.C. (1985) "Production of Biosurfactants by a Mixed Bacteria Population Grown in Continuous Culture on Crude Oil". Biotech Letters 7 (4), 217-222.

MunozCebrian J.M. (1983) "Microbiol Deterioration of Bituminous Materials: Selection of Microorganisms with Specific Metabolic Capacity on Hydrocarbons". Bioterioration 5, pp 472-485.

Olson G. J., Brinkman E. Iverson W.P. (1986) "Processing of Coal with Microorganisms", Electric Power Research Institute. Res. Project 2606-1.

Panchal C.J., Zajic J.E. (1977) "Isolation of Emulsifying Agents from a Species of Corynebacterium" Dev. Ind. Microbiol. 19 pp 569-576.

Roberts S.J., Hedrick H.G. (1978) "Degradation of Creosote Phenolic Compounds by Selected Bacterial Isolates" Dev. Ind. Microbiol. 20 pp 471-478.

Rosenberg et al (1979) "Emulsifier of Arthrobacter RAG-1: Isolation and Emulsifying Properties; Specialty of Hydrocarbon Substrate; Chemical and Physical Properties" Appl. Env. Microbiol. 37, pp 402-420.

Ryland L.B. Tamele Mw., Wilson J.N. (1960) "Cracking Catalysts" in Catalysis Volume VII Oxidation, Hydration, Dehydration and Cracking Catalysts ed. Paul H. Emmett, 1960, Reinhold Publ. pp 1-82.

Scott C.D., Strandberg G.W., Lewis S.N. (1986) Microbiol Solublization of Coal" Biotechnology Process 2 (3) 131-139.

Solig, Bens E.M., (1972) "Bacteria which Attack Petroleum Hydrocarbons in a Saline Medium "Biotech Bioeng 14, pp 319-330.

Vazquez-Duhalt R. Greppin H. (1986) "Biodegradation of Used Motor Oil by Bacteria Promotes the Solublization of Heavy Metals". The Science of the Total Environment 52, 109-121.

Walker J.D., Colwell R.R., Petrakis L. (1975) "Microbiol petroleum degradation; application of computerized mass spectrometry" Can J. Microbiol 21 1760-1767.

Walker J.D., Colwell R.R. (1976) "Long-chain n-alkanes occurring during microbiol degradation of petroleum degradation: application of computerized mass spectrometry". Can J. Microbiol 21, 1760-1767.

Walker J.D., Colwell R.R., Petrakis L. (1976) "Biodegradation rates of components of petroleum "Can J. Microbiol. 22 1209-1213.

Walker J.D., Colwell R.R. (1976) "Long-Chain n-alkanes occurring during microbiol degradation of petroleum". Cai J. Microbiol. 22, 886-891.

Walleston E.G., Haflin W.J., Ford W.D., D'Souza G.J. (1975) "FCC model valuable operating tool". The Oil and Gas Journal September 22, 1975 pp 87-94

Westlake D.W.S., Jobson A., Phillippe R., Cook F.D. (1974) "Biodegradability and crude oil composition". Can J. Microbiol 20, 915-928.

Witte M.K., Hoppe D.G., McCready R.G.L. (1986) "Microbiol Degradation of Carbonaceous Material in a Copper Molybdenum Concentrate" in Proc. 3rd Ann. Gen. Meet. Biominet, Tor. Can (ed) R.G.L. McCready, Canmet Special Publication SP86-9.

Zajic J.E., Supplison B. (1972) "Emulsification and Degradation of Bunker "E" Fuel Oil by Microorganisms". Biotech Bioeng 14 pp 331-343.

Zajic J. E., Supplison B. Volesky B. (1974) "Bacterial Degradation and Emulsification of No. 6 Fuel Oil", Env. SciTech 8 (7) pp 664-668.

RECOMMENDATIONS

The investigation into the biological decarbonization of the FCC catalyst will commence with a full characterization of the hydrocarbon (HC) contamination. This will include HC analysis, total organic carbon (TOC) and a C,H,O,N,P,S and Cl analysis. This will allow us to make decision concerning a viable method of employing either specific microbes on mixed populations, or both. Specific microbes with known capabilities may be purchased from commercial sources or obtained as gifts from other researchers, while mixed populations will be isolated from such places as sewage treatment plants and chronically (HC) - polluted environments. Alternatively, BCC may already have a culture that can successfully degrade the contaminated catalyst. In any case, the organisms will likely require a period of acclimatization, where the HC present will act to enrich for those organisms in a mixed population capable of degrading the HC, or for specific microorganisms a period of adjustment to relatively new substrate. If the HC contamination turns out to be very coal-like (coke), a fungus such as Polyporus versicolor or Candida sp. may be used, but if a broad spectrum of HC is present, a mixed population of bacteria and or fungi would probably work best.

To maximize the carbon degradation, optimal physicochemical conditions will be required. Optimal pH for most bacterial HC degradation has been reported as pH7 and if fungi are used, a more acidic pH will be necessary. Control of pH is best accomplished using buffers of known pH range while the use of pH meters will alow for a more exact monitoring. Temperature control should be relatively easy to maintain, as room or ambient temperature has usually been reported as acceptable. If the process requires more rigid temperature control an incubator could be implemented. The mineral nutrients could require the most attention. Nitrogen (N) and Phosphorous (P) are the most important with N supplied as nitrate and or ammonium and P as phosphate. Depending on the N and P content of the HC contaminant, the supplementation in molar quantities of either could have a significant effect on the degradation rate. The supplementation will be a one time initial additon in batch culline while if a continuous culture is employed dilution rates must be taken into consideration. A few examples of defined media used in the growth of bacteria or fungi on HC substrates are included in the appendix. Aeration, the supply

of oxygen, will be among the most important of parameters. If shake flasks are used, the baffled models are very efficient. Sparging the bioreactor will supply not only dissolved oxygen but also humid conditions. If a pure culture of microbes is used, an air scrubbing train of H_2SO_4 will be necessary to prevent airborne contamination. Mixed populations of unidentified taxa will not necessarily require sterile air. To optimize this HC degradation process, different physicochemical conditions can be implemented into a series of different flasks and monitored accordingly.

To follow the progress of the biodegradation of the FCC catalyst, a simple but efficient inexpensive method would be desirable. One method used by many investigators is to monitor CO_2 production caused by the mineralization of hydrocarbon substrates. This is accomplished by titration of a KOH (or NaOH) flask, of known normality, used for collecting CO_2 as carbonate, much like those shown in the appendix. Total organic carbon monitoring is feasible but the expense of daily monitoring would be unreasonable. Measurement of biological activity is also an indication of the overall ratio of degradation. In a mixed population, a variety of dehydrogenases will be produced, a method employing resazurin, an oxidation-reduction indicator, has feasibility as a monitoring agent.

Design and implementation of a suitable bioreactor will depend on the nature of success in the initial investigations. A few examples of bioreactors are given in the appendix. The most crucial parameter in choosing a bioreactor will be the methods of aeration and mixing, both important factors in aerobic hydrocarbon degradation.

P.D. Tackaberry
Project Scientist
Mineral Resources

G.M. Freeman
Operations Manager
Mineral Resources

V.I. Lakshmanan
Business Development Manager
Mineral Resources

APPENDIX: Agra Carbon-Degradation

MINERAL SALTS MEDIA FOR HYDROCARBON DEGRADERS

Bushell-Haas Medium

g/L		
	$MgSO_4\ 7H_2O$	0.2
	$CaCl_2$	0.02
	KH_2PO4	1.0
	K_2HPO4	1.0
	$NH4NO_3$	1.0
	$FeCl_3$	0.05

pH 7.8, mixed population

-pH 7 bacteria
-pH 5 fungi
-petri-plates prepared with 5% (v/v) hydrocarbon overlaid, or
-sterile filter paper soaked in HC laid on top.

Fedorak Westlake (1983)

g/L		
	NaSO4	2.0
	$MgSO4\ 7H_2O$	0.2
	K_2HPO4	0.5
	NH4Cl	1.0
	KNO_3	2.0
	FeSO4	trace

pH 7.4

- mixed population

AFNOR

pH 7.5

g/L:		
	$0.3(NH4)_2\ SO4$	membrane filter (0.2 uM)
	0.15 $NH4\ NO_3$	sterilize
	0.3 KH_2PO4	
	2.0 $Na_2HPO4.12H_2O$	mixed
	0.05 $MgSO4.7H_2O$	population
	0.05 $CaCl_2$-$2H_2O$	
	0.005 yeast extract	

Trace solution (1 mL)

1 mg $FeSO4$-$7H_2O$
1 mg $MnSO4$-H_2O
0.25 mg K_2MoO4
0.25 mg $Na_2\ B4O_7$-$10H_2O$
0.25 mg $Co(NO_3)_2$-$6H_2O$
0.25 mg $CuCl_2$-$2H_2O$
0.25 mg $ZnCl_2$
0.01 NH_4VO_3

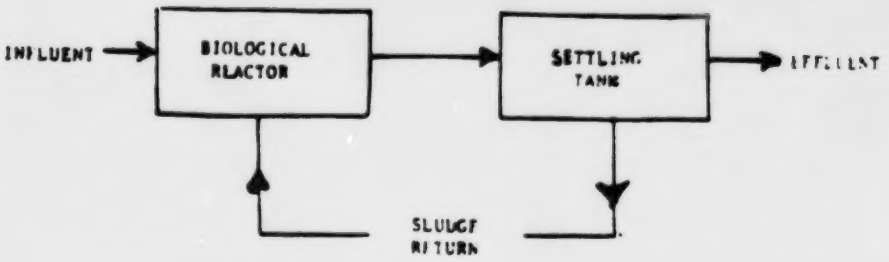
Zajic, Supplison (1972)

g/L

K_2HPO4	1.8
KH_2PO4	1.2
NH4cl	4.0
$MgSO4$-$7H_2O$	0.2
NaCl	0.1
$FeSO4$-$7H_2O$	0.01

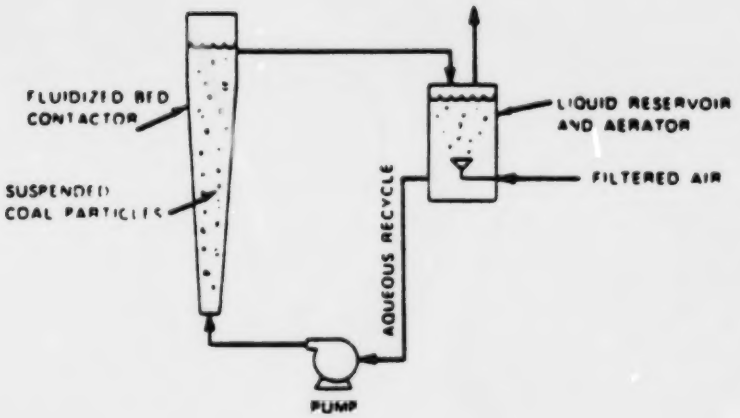
pH6

mixed population

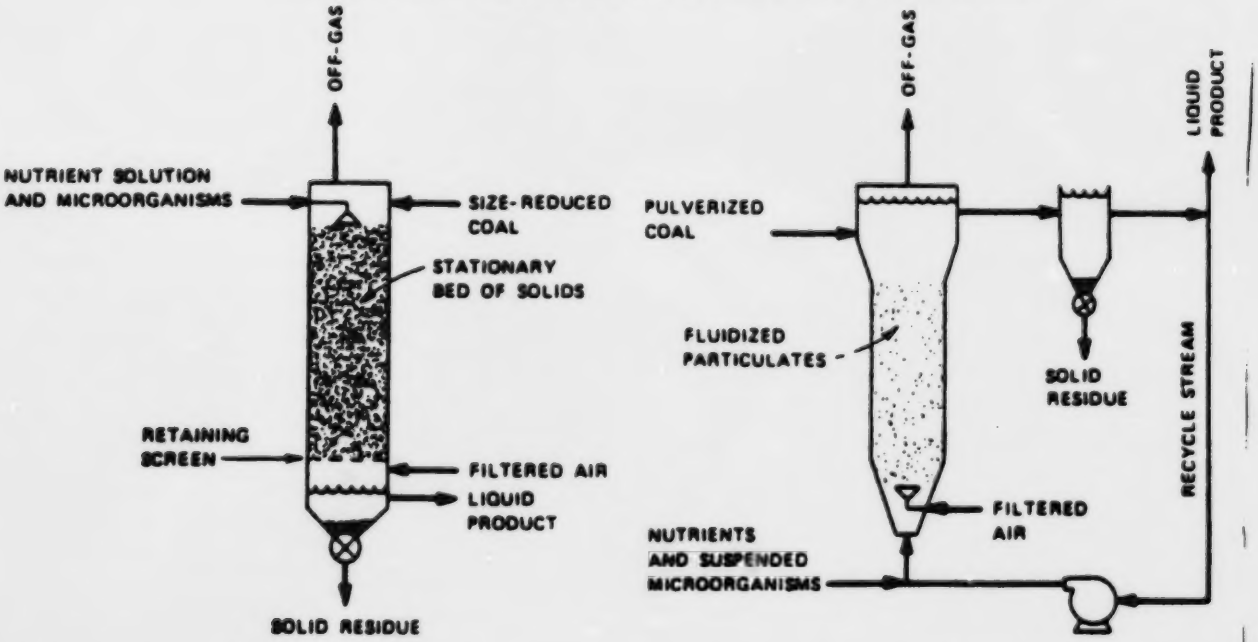


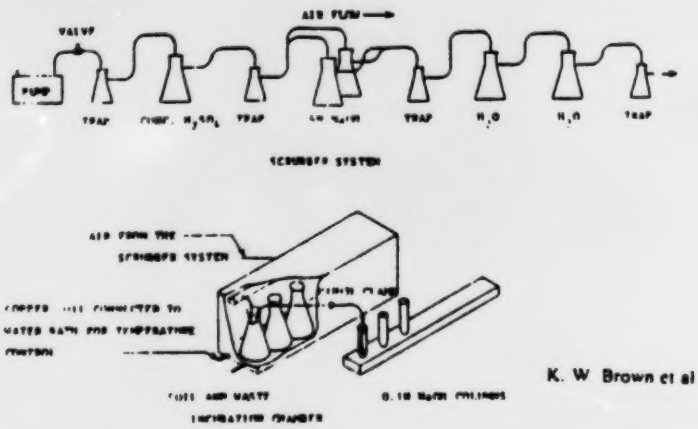
Experimental system for studying microbial attachment to coal particles while being fluidized in a tapered contactor.

ORNL DWG 85-1244



Two possible bioreactor configurations for the microbial solubilization of coal.





K. W Brown et al

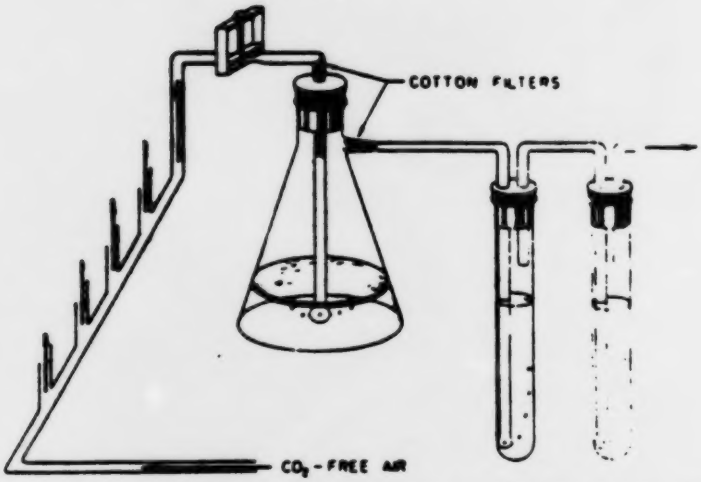
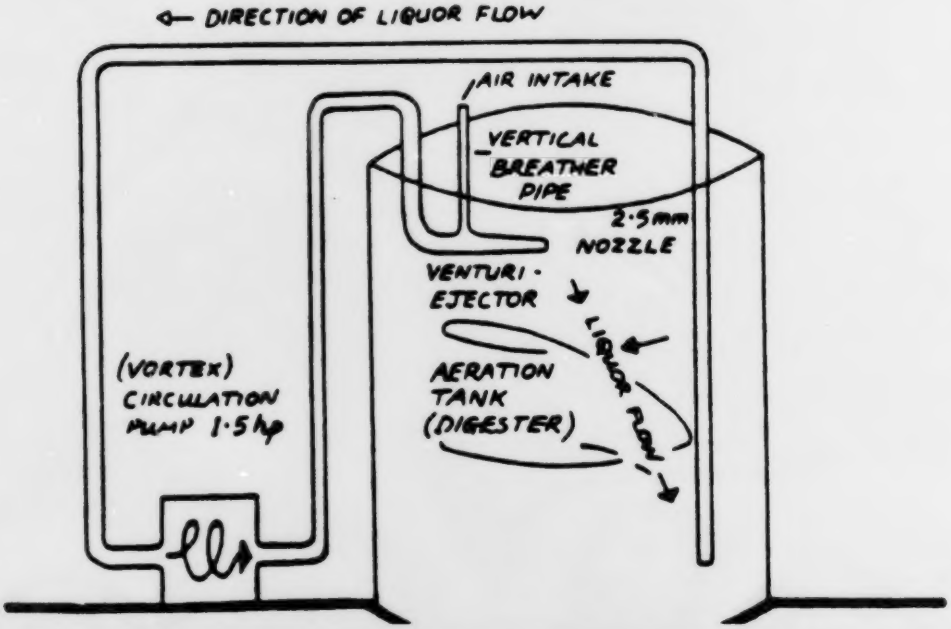


Diagram of a gas train for measurement of petroleum mineralization. CO_2-free air passes through a manifold, regulator, and cotton filter into a filter flask containing mineral solution, petroleum, and microorganisms. Evolved CO_2 is absorbed in 2 successive KOH-traps. The gas train is mounted on a rotary shaker.



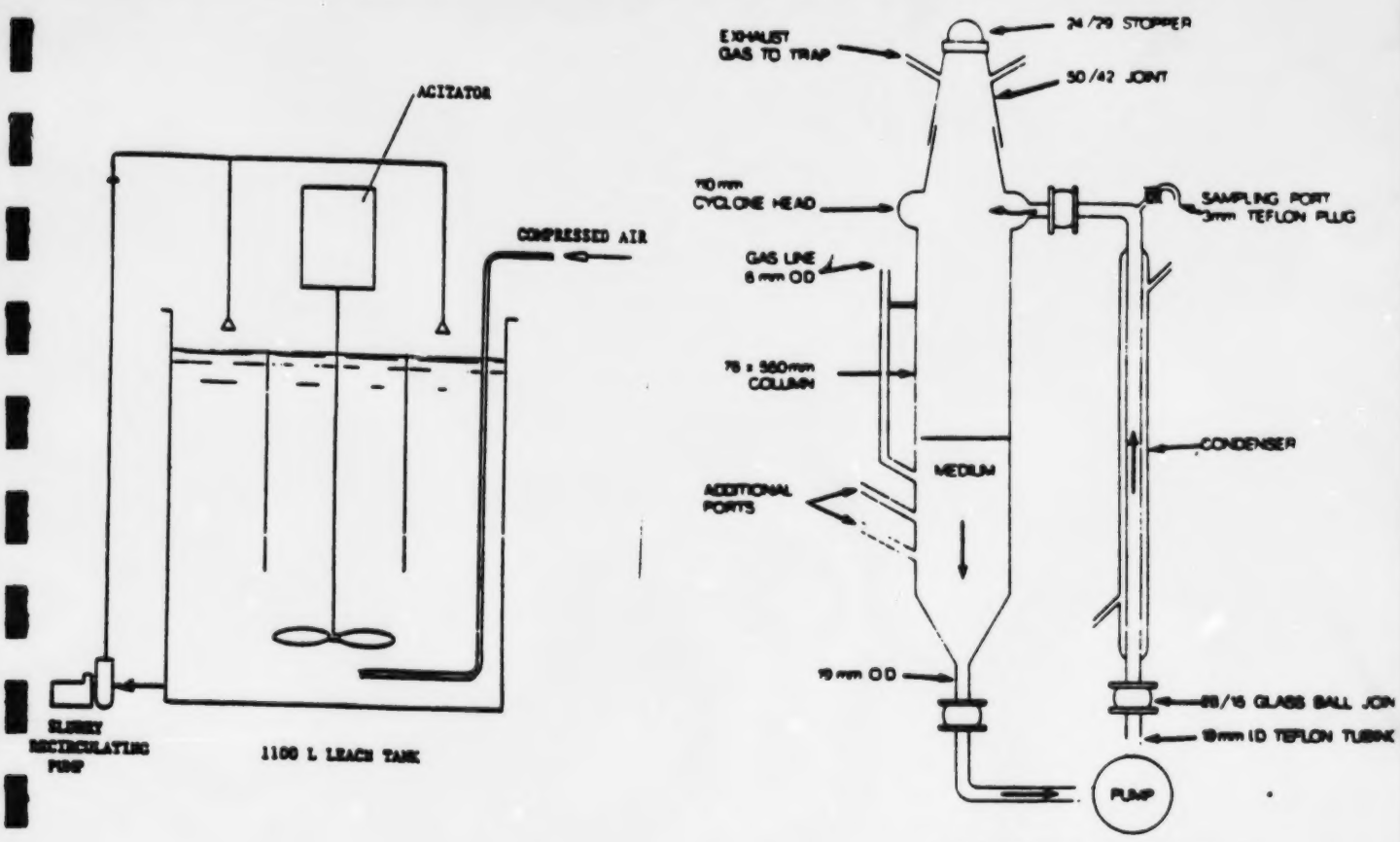


Diagram of cyclone fermentors.

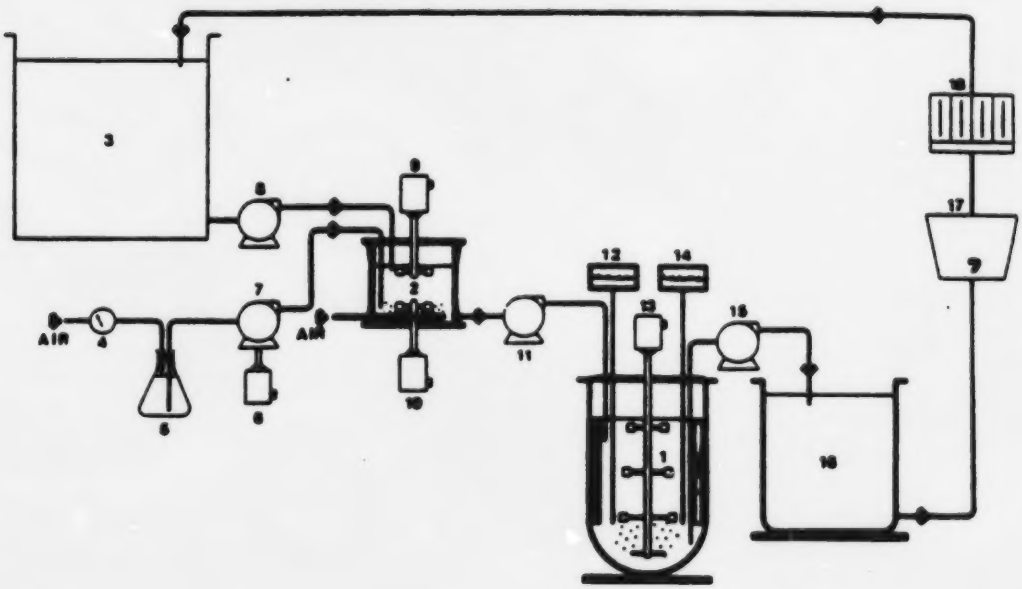


Fig. (1): Continuous culture device

1 - Growth fermentor
2 - "Emulsification fermentor"
3 - Medium reservor of fresh and used medium
4 - Manometer
5 - Crude oil flask
6, 9, 10, 13 - Motors
7, 8, 11, 15 - Pumps
12 - Control and registration of pH
14 - Control and registration of PO_2
16 - Storage tank of growth medium
17 - Centrifuge
18 - Filters

BIOLOGICAL CONSULTANTS Corp.

November 7, 1988

Ms. Caroline Frei
Cambrian Engineering
2200 Argentia Road
Mississauga, Ontario L5N 2K7
Canada

RE: Biological Decarbonization of Spent Catalyst
DSS Contract No. 23440-7-9224/01-SQ

Dear Ms. Frei:

Item 4.1 Literature Search. After reading the material contained within the attached bibliography there are several important items to note.

(1) Based on the number of articles and the diverse subject matter covered, a statement can be made that there is no indication that bacteria cannot function in the presence of substantial concentrations of heavy metals.

(2) A particular bacteria in a particular situation has a definite affinity for certain metals.

(3) There is a wide variety of environmental conditions which would affect the performance of individual or mixed culture bacteria.

(4) Little research of any quality has been performed on mixed cultures of bacteria.

(5) No documentation can be found with regard to the metal leaching capabilities of commercially available bacteria formulations.

(6) A perponderance of evidence demonstrates that hydro-carbons, in many forms, can be re-mediated.

Our literature search has confirmed a number of our original ideas.

(1) Heavy metals can be transferred from their carrier medium (oil) to water as part of the bacteria metabolitic functions.

(2) Bacteria themselves have several mechanisms with which they can accumulate, agglomerate, adsorb or physically retain heavy metals in or on their bio mass.

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(3) It is also evident that environmental conditions such as temperature pH, dissolved oxygen, sunlight, co-substrate and other factors affect the ability of bacteria to uptake or utilize heavy metals.

These conclusions or facts reinforce our original thoughts with regard to this project; that there is every likelihood that a commercially viable biological process can be developed in which the fluid cracking catalyst can be stripped of hydro-carbons and, that the potential is extremely high for the bio-accumulation of metals from this catalyst. While, to date, no research indicates that work has been done in this area, the papers dealing with similar or related subjects seem to substantiate these thoughts.

It is noted that there does not seem to be a straight forward approach to this since many of the bacteria that have the strongest potential for bio-accumulation of metals have little or no affinity for the remediation of hydro-carbon products. It would appear that a two or three step biological process would have the potential to provide this solution. We would first need to investigate the use of organisms already identified as having high metal accumulation characteristics in conjunction with known, tested and field proven commercial formulations that effectively deal with hydro-carbons.

Since this report is based on the Phase I Literature Search and that we have little or no characterization of the catalyst itself, we would feel comfortable in recommending further and more detailed lab work be performed to characterize the catalyst and to determine if, in fact, the metals that are bound in this silica matrix material are a.) strictly bound through an attraction to the silica material itself? b.) are they captured in a hydro-carbon substance which has agglomerated or attached itself through some chemical/physical bond to the catalyst material?

Attached is a list of candidate organisms that would be used in our initial screening of organisms to develop a commercially viable process for the recovery of metals from the FCC catalyst.

In conclusion, after an investigation of available literature, we find no indication that our original hypothesis regarding the ability of bacteria to remove hydro-carbons and to accumulate metals to be unsound. It would appear that based on the

literature available that this premise is still valid and should be relatively straight forward to accomplish, but will more than likely have to be emperically developed.

Should you have any questions with regard to any of the above, please feel free to call. This letter should also serve as the documentation needed to inform you that we have completed 100% of Item 4.1 with regard to the literature search. Thank you for your time and thoughtful consideration.

Sincerely,

R. W. Norcross, Jr. CHMM
President

RWN:pe

Enclosures

MESOPHILLIC BACTERIA

Corynebacterium equi

Arthrobactu simplex

Micrococcus luteus

Pseudomonas fluorescens

Pseudomonas stutzeri

Thiobacillus Ferrooxidans

Leptospirillium Ferrooxidans

Thiobacillus Thiooxidans

Thiobacillus Organoparus

Thiobacillus Thioparus

Alcaligenes Eutrophus

Pseudomonas Putida

Berjerinckia Lacticogenes

Pseudomonas Maltophilia

Staphylococcus Aureus

Nacardia Erythropolis

Neurospora Sitophila

Streptomyces Albus

Streptomyces Viridochromogenes

Actinomycetes Levoris

Bacillus Subtilus

Pseudomonas Aeruginosa

THERMOPHILLIC BACTERIA

Sulfobacillus Thermosulfidooxidans

Sulfolobus Acidocaldarius

Sulfolobus Brierleye

Sulfolobus Solfataricus

RESEARCH LITERATURE

Daniels, L. Bacterial methenogenesis & growth from C02 with elemental iron as the sole source of electrons. Science 237 (4814). 1987-509-11

Vazquez, Duhalt, R. Biodegredation of used motor oil by bacteria promotes the solubilation of heavy metals. Science of the Total Environment 52 (1-2). 1986.109-22

Dakajima,A. Selective accumulation of heavy metals by micro-organisms. Applied Microbiology & Biotechnology 24 (1). 1986.59-64

Hansen,C.L. Bacterial removal of mercury from sewage. Biotechnology & Bioengineering. 26(11). 1984.1.330-3

Chmiclowski, T. Bioaccumulation of germainium as a model for the nonspecific uptake of some metals by microorganism. Biochemistry of Soil Trans 9(2).1981. 306

Iverson,W.P. Microbial corrosion of metals. Advances in Applied Microbiology. 32(0). 1987.1-36

Thompson, L.C. Microbial degradation of metal cyanide complexes in mining waste. Abstracts - American Chemical Society 193(0).1987

Francis, A.T. Anaerobic bacterial dissolution of lead oxide. Archives of Environmental Contamination & Toxicology 15(6).1986.611-16

Slowik,T. Bioaccumulation of copper & lead by Sphaecotilus Natans. ACTA Microbial Pol 30(2).1981.183-94

Hill,RRH Bacterial bioabsorption of chromium from industrial cooling water. South African Journal of Science 82(10). 1986.595-96

Kasan,H.C. Bacterial bioabsorption of nickel from industrial cooling water. Environmental Pollution 48(4).1987.311-19

Lopes,CEA Absorption of metals by benthic microbial mats & sediments of Layoa Vernelha Brazil. Science of the Total Environment 58(1-2). 1986.55-62

Braynesteyn, A. Biological acid leach process US Patent 4571387. Office of Gas. US Patent Office Trademark. Office Patent. 1063(3).1986.1320

Rodriquez, M. Bacterial leaching of copper sulfide minerals by means of Thiobacillus Ferrooxidans. Revue of havinoamerican Microbiology. 27(3).1985.219-224

Bewley,RJF The prospects for land decontamination by microbial systems. Abstr. Pap American Chemical Society 194(0).1987

Kewen,H.C. Intracellula bioaccumulation of zinc by an Enterobacter -sp. Microbios 51(207).1987.89-96

Sulfita,T.M. Alternative nonlinear model for estimating second order rate coefficients for biodegradation. Applied Environmental Microbiology 53(5). 1987.1064-1068

Lester,T.W. Microbial accumulation of heavy metals in waste water treatment processes. Society for Applied Bacteriology Symposium Series 14(0).1985. 1415-1545

Hsich,K.M. Bioreactor for the study of deined interactrons of toxic metals & biofilm. Applied Environmental Microbiology 50(5).1985.1155-1161

Rudd,T. Complexation of heavy metals by extracellular polymerization of the activated sludge process. Journal Water Pollution Control Federation 56(12).1984.1260-68

Tates,M.V. Effect of microorganisms on in-situ uranium mining. Applied Environmental Microbiology. 46(4).1983.779-784

Schrader, M.E. Atomic absorption spectroscopic studies of molecular film absorption to metals from bacterial culture. Abstract American Chemical Society 183(0).1982.Coll-116

Moucawi,T. Biooxidation of added and natural hydrocarbons insoils effect of iron. Soil Biology & Biochemistry 13(5).1981.335-42

Ongorth, T.E. Pretreatment of industrial discharges to publicly owned treatment works. Journal Water Pollution Control Federation 52(8).1980.2246-2256

Senesi,D. Copper II complexes with refractory anions in surfaceants found in sewage sludge. Water, Air & Soil Pollutrons 35(1-2).1987.147-56

Saull,M.B. The effects of putrescidible domestic waste separation on selected plant characteristics. Int. Biodeterroratron 21(4).1985.295-300

Rubertiello, A. Nickel & vanadium as biodegradation monitors of oil pollutants in aquatic environment. Water Resources 17(5.1983.197-500

Rossin,A.C. The removal of nitrilodri acetic acid during biological sewage treatment and its effect on metal removal under shock loading. Environmental Pollution for a 30(1).1983. 1-26

Rodriguez-Leiva,M. Morphology of bacterial leaching patterns by Thiobacillus-Fernooxidans on oynthetic pyrite. Archives of Microbiology 149(5).1988. 401-5

Sugio,T. Existence of a new type of sulfite oxidase which utilizes ferric ions as an electron acepter in Thiobacillus-Ferrooxidans. Applied Environmental Microbiology 54(1).1988.153-6

Sugio,T. Synthesis of an iron-oxidizing system during growth of Thiobacillus-Ferrooxidans on sulfur-basal salts medium.Applied Environment Microbiology 54(1).1988.1150-2

Kasan,H.C. Intracellular bioaccumulation of zinc by an Enterobater. sp. Microbios 51(507). 1987.89-96

Obuekwe, C.T. Occurance of bacterial in pembina (North Central Alberta, Canada) Crude Oil pipeline systems and their potential role in errosion process. Applied Microbiology & Biotechnology 26(4). 1987.389-393

Jones,P.H. The effect of applied direct current on biological phosphorus uptake. Water Research 21(6).1987. 723-30

Ehrlich, H.L. What types of microorganisms are effective in bioleaching, bioaccumulation of metal ore, benefication & desulfurization of fossil fuels? Biotechnology & Bioengineering Symposium 16.1986.227-35

Brinckman, F.E. Chemical principles underlying bioleaching of of metals from ore and solid wastes and bioaccumulation of metals from solutions.Biotechnology & Bioengineering Symposium 16. 1986.35-44

Lundgren, D.G. Chemical reactions important in bioleaching and bioaccumulation. Biotechnology & Bioengineering Symposium 16.1986.7-22

Nakajima, A. Selective accumulation of heavy metals by microorganisms. Applied Microbiology & Biotechnology 24(1).1986.59-64

Thmiclowski, T. Bioaccumulation of germanium by pseudomonas putida in the presence of two selected substrates. Applied and Environmental Microbiology 51(5).1986. 1099-1103

Comeau,T. A biochemical model for biologically enhanced phosphorus removal. Water Science & Technology 17(11-12).1985.313-14

Lotter,L.H. The role of bacterial phosphate metabolism in enhanced phosphorus removal from the activated sludge process. Water Science & Technology 17(11-12.1985.127-138

Famari,B. Autoradiographic method to screed for soil microorganisms which accumulate zinc. Applied & Environmental Microorganisms 49(1).1985. 137-42

Hansen,C.C. Bacterial removal of mercury from sewage. Biotechnology & Bioengineering 26(11). 1984.1330-33

Ingledew, W.T. Ferrous iron oxidation by Thiobacillus-Ferrooxidans. Biotechnology & Bioengineering 16.1986.22-33

Torma,A.E. Bacterial Leaching. Prog. Ind. Microbiology
Bosecker,K. 16.1982.77-119

Smith,A.D. Stimulated Oil Biodegradation on Railway Tracks.
Hughes,R.. iodeterioration 5
Wood,B.J.B.

Heitkamp,MA Mineralization of Polycyclic Aromatic Hydro-
Cerniglia, carbons by a bacterium isolated from sediment
C.E. below an oil field. Applied Environmental
Microbiology 54(6).1988.1612-1614

Hutchins, Microorganisms in reclamation of metals.
S.R. Ann. Rev. Microbiol. 1986.40:311-36

Karavaiko, Microbiological processes for the leaching
Dr. G.I. of metals from ores. United Nations
Environment Programme 1-6976.1985.3-69

Saull,M.B. The effect of putrescible domestic waste
separates on selected soil characteristics.
International Biodeterioration 1985.
21(3).215-23

APPENDIX B

Biological Decarbonization of Spent Petroleum Catalysts

for

Cambrian Engineering Group Ltd.
2200 Argentia Road
Mississauga, Ontario
L5N 2K7

Attention: Caroline Frei

(DSS File No. 23440-7-9224/01-SQ)
ORTECH Acct. No. 41-10956

Phase 1 - Task 4.2

Catalyst Characterization

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Biological Decarbonization of Spent Petroleum Catalysts for Cambrian Engineering Ltd.

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Biological Decarbonization of Spent Petroleum Catalysts
for Cambrian Engineering Group Ltd.

1. INTRODUCTION

Catalyst characterization has been completed on a sample of hydrocarbon contaminated Nickel/Moly catalyst. The nickel/moly material consisted of dry dull black cylindrical extrusions, approximately 1/16" diameter and up to 1/4" long.

Analysis of the catalyst material included:

1) Total Kjeldahl Nitrogen
2) Hydrocarbon Characterization
3) Total Phosphorous, Sulfur and Chloride
4) Metal Concentrations
5) Total Organic Carbon

1) Total Kjeldhal Nitrogen

Results a) 0.26% (w/w)
b) 0.24%
c) 0.27%
$\bar{x}$ = 0.257%

The catalyst contains, on average, 2.57 g of nitrogen per kilogram sample.

2) Hydrocarbon Characterization

A sample of catalyst was soxhleted using methylene chloride and analyszed by gas chromatographic (GC) to allow quantitation of the chain length of the hydrocarbons present (12-28 carbon chains) as well as GC/mass selective detector which allows for greater separation and quantitation of a wide range of hydrocarbons and other compounds.

Included in this report are the profiles of the two detection methods.

Based on these profiles the following groups were used for identification and quantitation.

	% AREA
Aliphatic	45.6
Polyaromatic Hydrocarbons (PAH)	36.0
Sulfur-containing PAH	6.3
Unresolved hydrocarbons (aliphatic, cyclic and aromatic)	12.1

3) Total Phosphorous (P), Sulfur (S) and Chloride (Cl-)

a) petroleum ether extracted sample combusted in a Parr adiabatic bomb and determined using ion chromatography.

Cl- <100, <100, <100 mg/kg
P <50, <50, <50 mg/kg
S 370, 560, 310 mg/kg

b) room temperature deionized water, extracted and analysed for sulfate using ion chromatography

1260, 1320, 1600 mg/kg as S of water soluble sulfate

c) total oxygen bomb of whole sample: 5.51% Sulfur
8.36% Sulfur

4) Metal Concentrations

Analysis by DC plasma emission spectrophotometry.

Element	Concentration (% by weight)
Co	0.470
Cu	0.007
V	6.60
Mo	2.97
Ni	2.66
Cr	0.022
W	<0.001

5) Total Organic Carbon

following a sulfurous acid digest to remove CO_2

a) 19.7%

$\bar{x}$ = 19.8% (w/w)

b) 19.8%

note: graphitic carbon would be analyzed as organic carbon

2. CONCLUSION

Approximately 20% of the total contaminated catalyst has been determined to be organic carbon. Of this 20%, the organic carbon may be further classed as 45% aliphatics (C_{12}-C_{28}), 36% PAH's 6% Sulfur containings PAH's and a further 12% unresolved hydrocarbons.

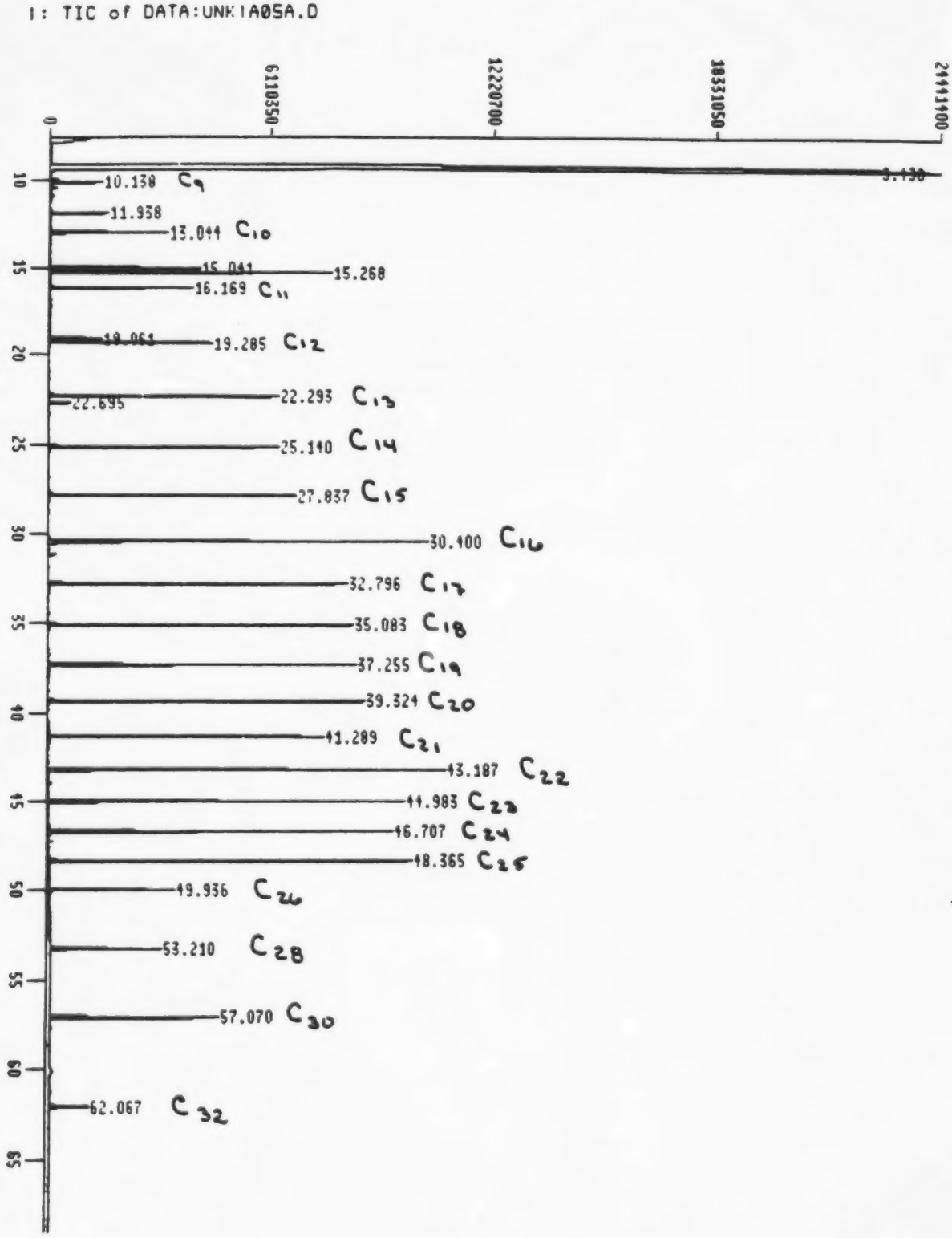
Elemental metals present as contaminants in high concentrations are Vanadium (6.6%) followed by Co (0.47%) and Chromuim, copper and tungsten in very low concentration. High nickel and molybdenum concentrations are considered to be present due to the Ni/Moly matrix of the catalyst.

Nitrogen at 257 mg/kg sample, Phosphorous at <50 mg/kg and Chloride at <100 mg/kg represents very low concentrations of these potential metabolic ions. Sulfur is present in considerably higher amounts being reported as high as 5500-8360 mg/kg sample. This could represent the fact that much of the metal contamination may be occuring as sulfides as well as the presence of S - containing PAH's.

P.D. Tackaberry
Project Scientist
Mineral Resources

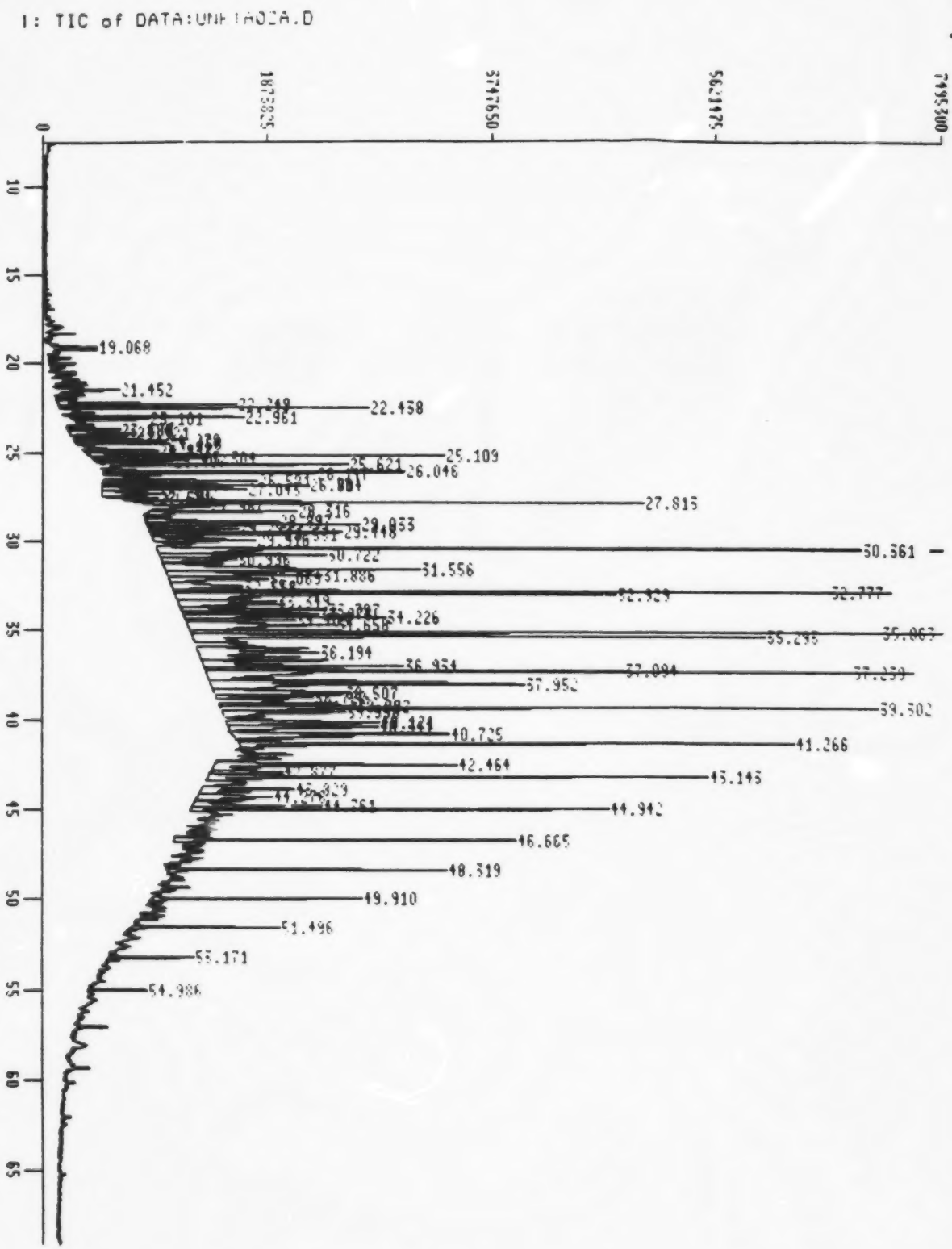
V.I. Lakshmanan
Manager
Mineral Resources

GC/MSD profile of standard



End of plot. Time = 7.63 to 69.00 minutes Chart speed = 0.32 cm/min

GC/MSD profile of sample



End of plot. Time = 7.53 to 69.01 minutes Chart speed = 0.32 cm min

APPENDIX C

BIOLOGICAL CONSULTANTS Corp.

March 6, 1989

Ms. Caroline Frei
Cambrian Engineering
Mississauga, Ontario
Canada

Re: (DSS File No. 23440-7-9224/01-SQ)

Dear Ms. Frei,

The following is a final report with regard to the Phase I, Biological Decarbonization of Spent Petroleum Catalysts, Task 4.3 and 4.4. This is a re-submittal, the original data numbers were transposed by a mistake during preparation and we did not catch them until now. Results and conclusion are uneffected.

The results of our labwork are middle of the road. The results are neither discouraging, or extremely encouraging. The results are based on a methodology that was not originally envisioned when our initial proposal for this work was submitted. As you recall, there were some arbitrary budget cuts made for the submittal to DSS. Unfortunately, the budget we had originally submitted was tight, and took into consideration, not only the biological treatability of the catalyst, but also dealt with the ability of commercial bacteria and natural occurring organisms to uptake certain metals. However, due to the extreme reduction in the proposed budget, the metalwork could not be accomplished due to the expense of GC/ Mass spec work.

We did, in fact, conduct the treatability phase, but since our funds were so limited, we had to develop a testing program that fit the budget, rather than the project objectives. To accomplish this within the restraints of our budget, we tried to determine the amount of organic reduction by weight.

We did determine that the catalyst was only slightly bioinhibitory; however, there is not a great deal of organic matter present. The analytical data has shown approximately 20% organic carbon. However, it also appears that a very large percentage of this 20% carbon is graphitic carbon which is not biodegradable.

The probable chemical structure shown in the analysis of the catalyst can be readily biodegraded by the commercial cultures that we used, yet very little weight-loss occurred. This would

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Indicate that there is very little true organic carbon present, or that the graphitic carbon is providing some sort of protective coating to the organics.

We did preliminary work using solvents to remove the hydrocarbon mixture from the catalyst. Methylene chloride was used in a soxhleted extraction for the analytical determinations. It was felt that this would not be practical on a commercial scale, and disposal of the solvent could be become a problem into itself. Alcohols, keytones, and aliphatic hydrocarbons did not give us satisfactory results.

In conclusion, our original thoughts are still viable.

1. The FCC catalyst is not bioinhibitory.
2. Bacteria should be able to uptake metals.
3. A suitable solvent may be useful to a biological solution.

There would appear to be more work to be done on this initial phase to develop a better base line answering questions such as:

1. What is causing the slight inhibition of biological growth?
2. What effect is the graphitic carbon having on the reaction?
3. Can better removals and uptake be seen when the biological degradation is conducted in the presence of a good stripper/solvent material?
4. How will the introduction of non-commercial organism effect the overall process?
5. How much better definition of the process could we obtain with greater funding for analysis of the process?

Sincerely,

R.W. Norcross, Jr./ CHMM

RWN/cl

BIODEGRADATION FEASIBILITY STUDY FOR A PETROLEUM CATALYST

OBJECTIVE: The objective of this study is to ascertain the possibility and/or extent of organic removal from spent petroleum catalyst. The assessment of organic removal was measured by weight reduction.

MATERIALS AND METHODS: The spent catalyst was examined as to its physical characteristics upon arrival in the laboratory. The catalyst was segregated by size so that the larger particles were segregated from the "fines" generated during shipment. It is the larger particles that were treated in this experiment.

The segregated larger particles were placed into an oven at 103^0 C for a period of three days to remove any excess moisture that the particles may have absorbed.

The dried particles were then placed into dried, tared Erlenmeyer flasks and weighed to the nearest 0.1 mg. A separate flask was prepared for each formulation tested. Nutrients were weighed into each flask and 100 ml of water was added to create an aqueous environment for the microorganisms.

An aliquot of presoaked organisms was then added to the corresponding flask and the flasks were placed onto an orbital shaker at 100 rpm for sixty days. An uninocculated control flask was also prepared in the same manner with the exception of the inocculation step.

A standard plate count was conducted on the Advance Bio Cultures L-104 inocculated flask after 24 hours to assess whether or not the catalyst exhibited any toxicity toward the inocculum.

After it was determined that the sample was not inhibitory or toxic to the inocculum the samples were allowed to incubate for the sixty day period. At the end of that period the flasks were removed from the orbital shaker and placed into a drying oven at 103^0 C until a constant weight was measured for each flask.

The final weight of each flask was compared with the initial weight of the flask and a weight loss was calculated. The weight loss in the uninoculated control was compared to the weight loss in the inocculated flasks and a percent degradation was obtained.

DATA: The sample was placed in the oven at 103^0 C on 10/01/88 where they remained for a period of three days.

The sample was removed from the oven on 10/04/88 and placed into a drying chamber until cooled.

The sample was divided into aliquots and added to tared, dried 250 ml Erlenmeyer flasks and nutrients were added. The raw data follows:

	TARE	TARE + SAMPLE	MSP/AS
CONTROL	131.5991	169.6394	170.7318
I-107	138.6731	168.6142	169.6817
L-103	138.8606	172.1453	173.2324
L-104	128.6658	160.9845	161.9741

The MSP/AS is the additive total of the Monosodium phosphate and Ammonium sulfate added to each flask.

To the flasks with a product designation, 1 ml of 1/10 dilution of Advanced Bio Cultures was added.

To account for possible weight gain due to the inoculum, another dried, tared flask was prepared with no sample, just nutrients and inocculum. The data are as follows:

	TARE	MSP/AS
CONTROLB	130.8035	131.8035

Once the needed flasks had been prepared, 100 mls of deionized water was added to each flask.

The flasks were placed upon an orbital shaker at 100 rpm at room temperature.

After 24 hours on the orbital shaker, a standard plate count was performed upon one the L-104 inocculated flask.

The plate was read after being incubated for 24 hours at 35^0 C for 24 hours. The result is reported below.

The physical appearance of each flask was monitored throughout the experiment. After the first day the appearance any of the flasks did not change. The first days observations are as follows:

ODOR: CHARACTERISTIC: PETROLEUM
INTENSITY: VERY SLIGHT

APPEARANCE: CHARACTERISTIC: Inoculated samples have slight turbidity due to inoculum growth. Sample is at bottom of flask.

Fines from the sample are free and settle upon termination of agitation.

No. of Phases: 2 COLOR: Black

These observations were the same until termination of the experiment.

Upon termination of the agitation the flasks were placed into a drying oven at 103° C until dried to a constant weight. The weights for each flask were then recorded and compared to previous weights to calculate a percent loss. The final weights were as follows:

	FINAL WEIGHT
CONTROL	171.5773
I-107	170.4636
L-103	173.9900
L-104	162.4693
CONTROL B	132.3052

It is apparent from the raw data that there was little or no change in the weight of the samples unless the weight added due to inoculum is backed out of the final weights of the inoculated flasks. Since the weight due to inoculum is 0.5017 grams it represents a significant addition of weight.

RESULT: The standard plate count for the L-104 inoculated flask after 24 hours of incubation was 60×10^4 cfu/ml. This indicates that the sample is slightly inhibitory but should be able to work effectively given proper time.

The L-104 formulation was chosen as the candidate formula because it has historically been the best performer. Also it appeared from a visual standpoint to be the most promising.

The recorded weight loss shows no significant differentials and were of little help in determining the best formulation.

The weight loss after the sixty day period was found to be minimal indicating that little or no degradation was occurring on the particles. The aqueous phase of the treated samples did not have any indication of an emulsion of extensive cloudiness that would indicate significant degradation was occurring.

Biological Decarbonization of

Spent Petroleum Catalysts

(DSS File #23440-7-9224/01-SQ)

Phase 1
Final Report
Task 4.1 to 4.4

P.D. Tackaberry
V.I. Lakshmanan
Mineral Resources

for

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April 12, 1989

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1.0 INTRODUCTION

A DSS sponsored project, (DSS #23440-7-9224/01-SQ) "The Biological Recovery of Metal Values from Spent Catalyst", was undertaken by ORTECH International as part of a joint program established by Agra Industries Ltd. The project was discontinued by the sponsor on March 8, 1989 and all work was terminated as of March 31, 1989.

This report will describe the contract work ORTECH has completed or partially completed as of the termination date. Because the literature search and catalyst characterization have already been documented, and submitted to Cambrian Engineering Group Ltd., only tasks 4.3 and 4.4, the pretreatment and comparison tasks will be reported on.

2.0 OBJECTIVE

The objective of the studies undertaken by ORTECH as part of Phase 1 of this project were to optimize biological decarbonization of spent petroleum cracking catalyst. This was accomplished through 4 different tasks (4.1 to 4.4) as prescribed in the original proposal.

A thorough literature search (task 4.1) was conducted to review existing methodologies and to plan an acceptable course of action. Chemical and physical characterization of the catalyst was undertaken (task 4.2) to maximize the optimization process (task 4.3). Following various treatments of the catalyst, a comparison was made (task 4.4) to the usual thermal regeneration step.

3.0 MATERIALS AND METHODS

3.1 ATTRITION

Autogenous grinding was performed on < 200 g samples using a rubber-lined grinding mill on a simple laboratory roller. The catalyst was processed for a maximum of 4 hours at 70 rpm and then classified using a 30 mesh screen. The (+30) fraction was kept as the attritioned sample; the (-30) fraction as the fines.

3.2 BIODEGRADATION

3.2.1 Culture

A culture formulation supplied by Biological Consultants Corporation (BCC) to ORTECH was described as "formulation L-104" Advanced Bio Cultures manufactured by Solman Corp., Orange, California. It was prepared by presoaking 4-12 hours the dehydrated culture in warm water at a concentration of 120 g per litre.

3.2.2 Basal Media for Optimization Tests

Two different mineral salts formulations were used based on work by Zajic (1972) and by Fedorek and Westlake (1984):

Zajic Salts (Z-salts)	(g/L)	Fedorak-Westlake (F/W salts)	(g/L)
K_2HPO_4	1.8	Na_2SO_4	2.0
KH_2PO_4	1.2	$MgSO_4 \cdot 7H_2O$	0.2
NH_4Cl	4.0	K_2HPO_4	0.5
$MgSO_4 \cdot 7H_2O$	0.2	NH_4Cl	1.0
NaCl	0.1	KNO_3	2.0
$FeSO_4 \cdot 7H_2O$	0.0.1	$FeSO_4 \cdot 7H_2O$	trace
pH6		pH7.4	

The media was used unsterilized because of the non-aseptic conditions used with the bioculture and new material catalyst. Distilled water was used as the aqueous.

Sodium lignin sulfonate (NaLS) was used as a surfactant (Liu et al., 1981) and incorporated into the aqueous testing at [100 mg/L] either alone or with full strength F/W salts solutions (NaLS/FW).

3.3.3 Culture Conditions

Six different aqueous treatments of the catalyst were used in these optimization tests:

1) Z-salts (Zajic mineral salts) plus L-104.
2) F/W salts (Fedorate-Westlake mineral salts) plus L-104.
3) NaLS (Sodium lignin sulfonate).
4) NaLS/FW plus L-104.
5) NaLS with inoculum (L-104) present.
6) H_20

Testing consisted of 100 mL of aqueous in a 500 mL Erlenmeyer flask agitated on a wrist shaker so that the medium would rise to 2/3 the height of the flask. Those tests with inoculum had 10 mL of [120 g/L] L-104. Thirty gram (approx) samples were added and incubated for 7 days at room temperature (~23°C).

The Erhenmeyer flasks were fitted with rubber stoppers and glass tube openings. Distilled water was added on Day 4 to correct to 100 mL volumes. Seven-day incubation periods were used on the advice of the Scientific Authority* in the consideration that any longer a time period would be impractical.

3.4 FILTRATION

Upon completion of 7 days incubation, the flask contents were vacuum filtered through Whatman #1 qualitative paper in a Buchner funnel. The precipitate was washed with one to two volumes of distilled water and further oven-dried at 100°C for 24 hours.

* (Dr. R.G.L. McCready, CANMET)

3.5 ANALYTICAL

3.5.1 Soxhlet Extration

Approximately 15 gram samples were weighed to the nearest 0.1 mg before and after extration. The soxhlet apparatus ie, the thimble set-up and the flask, were weighed to the nearest 0.1 mg before and after extraction. as well. Extraction took place using approximately 125 mL hexane at a rate of 20 cycles per hour for 4 hours.

Following this, the solvent was distilled in a rotary evaporator. The remaining extract and the thimbles with samples were oven dried at 105°C for 6 hours before gravimetric determinations.

3.5.2 Loss on Ignition

This analysis was conducted in an attempt to compare weight losses of the samples using high temperatures, as in normal thermal regeneration. Crucibles were prepared in a 1000°C oven to remove all organic traces and allowed to condition in a dehydrating chamber. Samples were conditioned in the dehydrating chamber and weighed in the crucible to the nearest 0.1 mg. The samples and crucible were then subjected to 1000°C for 1 hour then removed to a dehydration chamber for cooling.

The samples and crucibles were then weighed to the nearest 0.1 mg and the difference between before and after LOI reported.

3.6 REAGENT PURITY

All chemicals used were of analytical reagent grade and prepared with distilled water.

4.0 EXPERIMENTAL PROGRAM

The results of the scoping tests by BCC suggested very limited (<0.05%) carbon degradation due to the action of microorganisms. A formulation of microorganisms L-104, was, however, presented to ORTECH by BCC for the optimization program. In order that optimization be given the opportunity to succeed, a variety of media were designed based on hydrocarbon fermentation quoted in the literature. This approach was incorporated into the suggestion that, based on BCC findings, an attempt to be made to remove the graphitic carbon. Attrition was recommended as the best method of accomplishing this.

4.1 CATALYST ATTRITION

Four hours of attrition resulted in a maximum of 7.4% weight loss, producing a shiny black catalyst particle (+30 mesh) and dull black fines (-30 mesh) (Table 1). This is in contrast to the dull black-brown appearance of the as received catalyst.

An initial soxhlet hexane extraction of the as received catalyst vs the attritioned catalyst resulted in sample weight loss percentage of 4.2% and 5.45% respectively (Data not shown). Although unconfirmed by repeated testing (low supplies), these results indicate a greater amount of organic carbon available to hexane extraction following attrition. The flask extract did not support this claim (3.8% and 2.5% resp.), and was considered not as reliable as actual sample weight loss.

Since more organic carbon seemed to be available after attrition, a greater number of tests were performed on the attritioned samples. Generally these tests resulted in higher sample weight loss percentages than similar tests on as received samples.

4.2 AQUEOUS TREATMENTS

Gravimetric determinations made on the various aqueous treated catalyst samples, before and after hexane soxhlet extraction, showed that the attritioned samples generally had higher weight loss percentages than the "as received" samples (Table 2). The exceptions to this generalization were the Na-lignin sulfonate/Fedorak-Westlake formulation and the H_20 treatments where the "as received" samples had higher weight loss percentages.

If greater weight loss percentage indicates greater amounts of organic carbon associated with the catalyst, the following observations may be made. L-104 inoculated media formulations represented those tests of the attritioned catalyst which had the greatest sample weight loss percentages. It is therefore possible that the L-104 inoculum and its associated formulation is interfering with accurate determinations. Alternatively, the large amount of bound graphitic carbon on the "as received" catalyst, could be preventing the biological or non-biological leaching of the organic carbon.

The fact that sample weight loss percentages were lower with Na- lignin sulfonate, a surfactant, may indeed result in more organic carbon being leached into the medium using either type of catalyst.

The organic extract flask residues following hexane soxhleting produced similiar results for the "as received" sample weight losses. However, the same cannot be said for the attritioned samples flask extracts. The H_20 treatment consistently yielded the least weight loss percentages followed by the inoculated media formulations, in considerably different increasing order than the sample weight losses.

4.3 LOSS OF IGNITION (LOI)

The largest weight loss percentages occured with the Na-lignin sulfonate (NaLS) and the NaLS/Fedorak Westlake inoculated formulations of attritioned catalyst. The control (no aqueous) untreated samples were less than these values while the Fedorak-Westlake salts, Zajic salts, H_20 and NaLS treatments had similar or lower values. The "as received" samples were all relatively similar in weight loss percentage.

The results indicate extreme variability in this test method. It was thought that the untreated control samples (no aqueous, Table 2) would have had the largest weight loss percentages. However, a possibility does exist that, as indicated by the extraction data, the organic carbon is not degraded, biologically or otherwise, to any significant extent. Therefore, LOI results may be variable due to the aqueous treatment only and not the effect on carbon removal.

5.0 CONCLUSION

The optimization program conducted by ORTECH was inconclusive insofar as obtaining an optimal medium formulation of organisms, mineral salts, and or surfactant for decarbonization of this catalyst. Because no clear trends emerged from this set of experiments, it may indicate that the catalyst is relatively recalcitrant to biological decarbonization.

For this program to be more successful the following recommendations may be made based on the findings of the literature review and of this report.

1) Evaluate non-commercial microorganisms such as those found associated with existing oil refinery sludge ponds.

2) Conduct catalyst attrition to remove graphitic and other loosely bound carbon.

3) Investigate biological and non-biological surfactants as decarbonization agents.

P.D. Tackaberry
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Manager
Mineral Resources Centre

6.0 REFERENCES

Zajic, J.E., Supplison, B. (1972) "Emulsification and Degradation of Bunder "C" Fuel Oil by Microorganisms. Biotechnology and Bioengineering, 14, 331-343.

Fedorak, P.M., Westlake D.W.S. (1984), "Degradation of Sulfur Heterocycles in Prudhoe Bay Crude Oil by Soil Enrichments." Water, Air and Soil Pollution, 21, 225-230.

Norcross, R.W. (1989), "Biodegradation Feasibility Study for a Petroleum Catalyst." Biological Consultants Corporation Report for DSS File #23440-9-9224/01-SQ), March 6, 1989, January 3, 1989.

Liu, D., Strachan, W.M.J., Thomson K., Kwasniewska, K., (1981), "Determination of the Biodegradability of Organic Compounds." Environmental Science and Technology, 15, 788-793.

	Attrition Time (Hours)					
	0	0.5	1	2	3	4
SAMPLE WEIGHT (g)	147.2586	141.5081	139.9515	138.3471	137.0962	136.3339
TOTAL %	0	3.9	4.9	6.1	6.9	7.4

TABLE 1: Effect of Attrition on Weight of Catalyst

		AS RECEIVED CATALYST % Weight Loss of Sample			ATTRITIONED CATALYST % Weight Loss of Sample		
		LOI	SOXHLET Actual sample	Extract/sample	LOI	SOXHLET Actual sample	Extract/sample
Z salts	1	27.5	3.5	3.3	2.0	3.7	3.6
	2					6.5	4.0
F/W salts	1	21.3	4.2	3.5	19.7	3.8	3.6
	2				26.8	6.4	3.5
NaLS		19.0	3.7	3.3	19.8	5.0	4.0
NaLS with inoculum					24.7	6.9	3.7
NaLS & FW salts	1	25.5	12.4	3.9	21.5	6.3	3.7
	2				3.5	11.9	3.5
H_20		20.0	3.9	3.7	19.4	2.2	2.9
No Aqueous		24.0	3.6	2.7	23.0	4.1	3.7
Fines	1				35.0	6.9	3.7
	2				34.6	0.3	2.9

TABLE 2: Effect of Medium Formulations on Sample Weight Loss Percentage